

A focus on PAT in freeze-drying



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Overview

- Freeze-drying – Process steps and questions?
- QbD in Freeze-Drying
- Freezing Stage
 - Critical process parameters : solidification end point, eutectic formation, glass formation
 - PAT in the freezing Stage (development & production)
- Primary Drying Stage
 - Critical process parameters : collapse temperature, drying rate, drying end point
 - PAT in the 1ry drying Stage (development & production)
- LyoDEA – A new PAT



Problems with lyophilizing biologicals

Biologicals are labile, complex, often multi-domain macromolecules

- Freeze drying can strip stabilising water from proteins
 - requiring careful excipient choice to maintain activity
- Freeze-concentration may induce unfolding and aggregation
 - Specific electrolyte balance required to control the weak forces
- Final product storage stability may be problematic (cold chain)
- Instability on reconstitution
 - May require chilling during dispensing to minimise degradation
 - May suffer from aggregation and denaturation
- May lose active material by non specific adsorption to glass/metal/plastics

So process optimisation is a key issue for manufacturers whose processes include freeze drying.



QbD in Freeze Drying

- Identify Critical Quality Attributes for the freeze dried product
 - Stability, Reconstitution time, cake structure, mechanical strength
- Determine critical thermal properties of the product
 - Eutectic temperatures, glass transition (T_g) and collapse temperature (T_c)
- Develop & implement in-line PAT tools to monitor the process
 - Freezing onset, freezing rate, amount of ice formation, solidification end point
 - Primary drying rate and end point
- Use DoE to establish the Design Space for safe operation
 - Assess the impact of product, formulation, container and dryer
 - Develop models for each stage of the process to assess the impact of changes
 - Risk assess the impact of excursion outside of this space (product & Equipment failure)



Process Analytical Technologies

- A system for **designing, analyzing, and controlling manufacturing** through timely measurements (i.e. during processing) **of critical quality and performance attributes** of raw and in-process materials and processes with the goal of ensuring final product quality

In the PAT framework (FDA September 2004) these tools can be categorized according to the following:

- Multivariate tools for design, data acquisition and analysis
- **Process analyzers**
- **Process control tools**
- Continuous improvement and knowledge management tools

<http://www.fda.gov/downloads/Drugs/Guidances/ucm070305.pdf>



PAT in production : The expectations are high

Global load monitoring	As freeze-drying is dependent upon heat and mass thermal transfer, some heterogeneities may limit control . It may be erroneous to rely on individual vial measurement to control the whole load.
loading/unloading	Compatibility with automatic loading/unloading devices. The placement and removal of vials must not be impaired.
CIP & stoppering	Compatibility with cleaning in place(CIP)/stoppering devices. No leads should compromise the movement of shelves, CIP ramps, or nozzles
Aseptic handling compliance	Compliance with aseptic handling. There should be no source of contamination within the materials or during positioning
Steam sterilization	Steam sterilization. The device must sustain repeated steam sterilization at a minimum of 123 °C and 2 bars for a duration of 3 h.
Leakage control	Placement of the device should not induce freeze-dryer leakage. It should also support at least a 5 microbar vacuum, and measurement should be independent of equipment leak rate.
Integration	Simple integration into an industrial freeze-dryer. The device should be installed to current existing ports using tri-clamp flanges, and the data acquisition signal should be compatible with 21CFR Scada / recorders



Comparison of PATs for Production

Drying rate & End Point

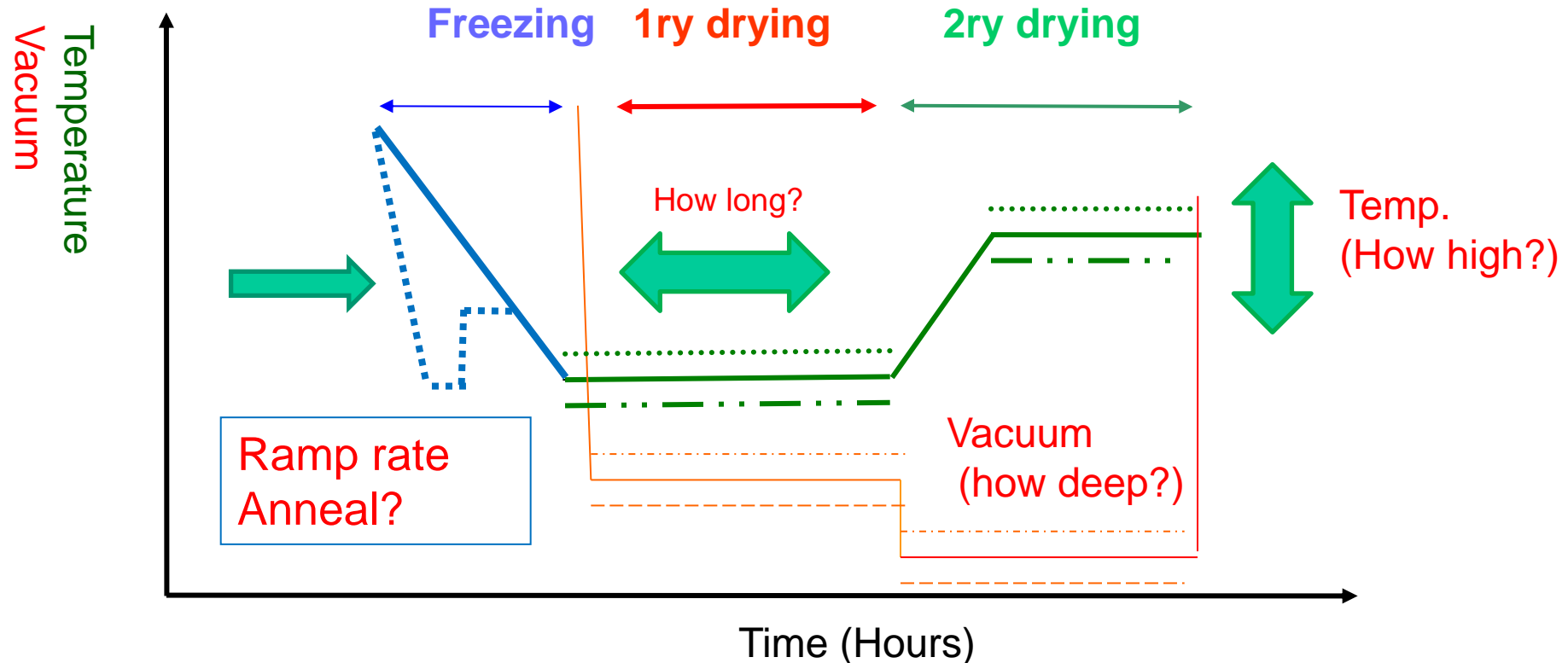
End point

Success Factor	Monitor Global Load	Automatic Loading	CIP + Stoppering Device	Aseptic Handling	Steam Sterilizable	Leak Rate Control	Simple Integration	Calibration
Temperature probe	NO	NO	NO	+/-	YES	YES	+/-	YES
Wireless product temperature probe	NO	NO	NO	+/-	YES	YES	+/-	YES
Conductivity probe	NO	NO	NO	NO	YES	YES	YES	YES
Microbalance	NO	NO	NO	NO	NO	+/-	NO	YES
FTNIR product probes	NO	NO	NO	NO	YES	YES	NO	YES
Pirani/capacitive differential pressure control	YES	YES	YES	YES	+/-	YES	YES	YES
Moisture probe	YES	YES	YES	YES	NO	YES	YES	YES
Pressure rise measurement	YES	YES	YES	YES	YES	NO	YES	NO
Mass spectrometry	YES	YES	YES	YES	NO	NO	NO	
TDLAS	YES	YES	YES	YES	YES	YES	NO	
Cold plasma	YES	YES	YES	YES	YES	YES	YES	

Modified from Mayeresse et al. PDA J Pharm Sci Technol. 2007 May-Jun;61(3):160-74



Freeze drying cycle design



- Process design achieved by multiple cycles to establish high&low operational limits
- Repeatability established by consistency of batches and process trend monitoring



PAT : What do you need to measure and at what scale

Product Scales

- 1: **Microscopic** : Molecular dynamics in the unfrozen phase (relevance to collapse temperature)
- 2: **Mesosopic**: Ice crystals and connectivity (relevance to drying rates)
- 3: **Macroscopic I** : Ice formation from the base (impact on scale 2).
Temperature differences across the ice layer, changing ratio of ice layer and dry layer during drying,
- 4: **Macroscopic II** : design of vial (size, wall thickness, base characteristics),
Impact of vial dimensions in relation to fill height, clustering of vials

Engineering scale

- 1: size of shelf, separation of shelves, edge effects
- 2: loading of drier, condenser capacity, coolant capacity, dimensions of ducting between the chamber and condenser (choke flow) etc..

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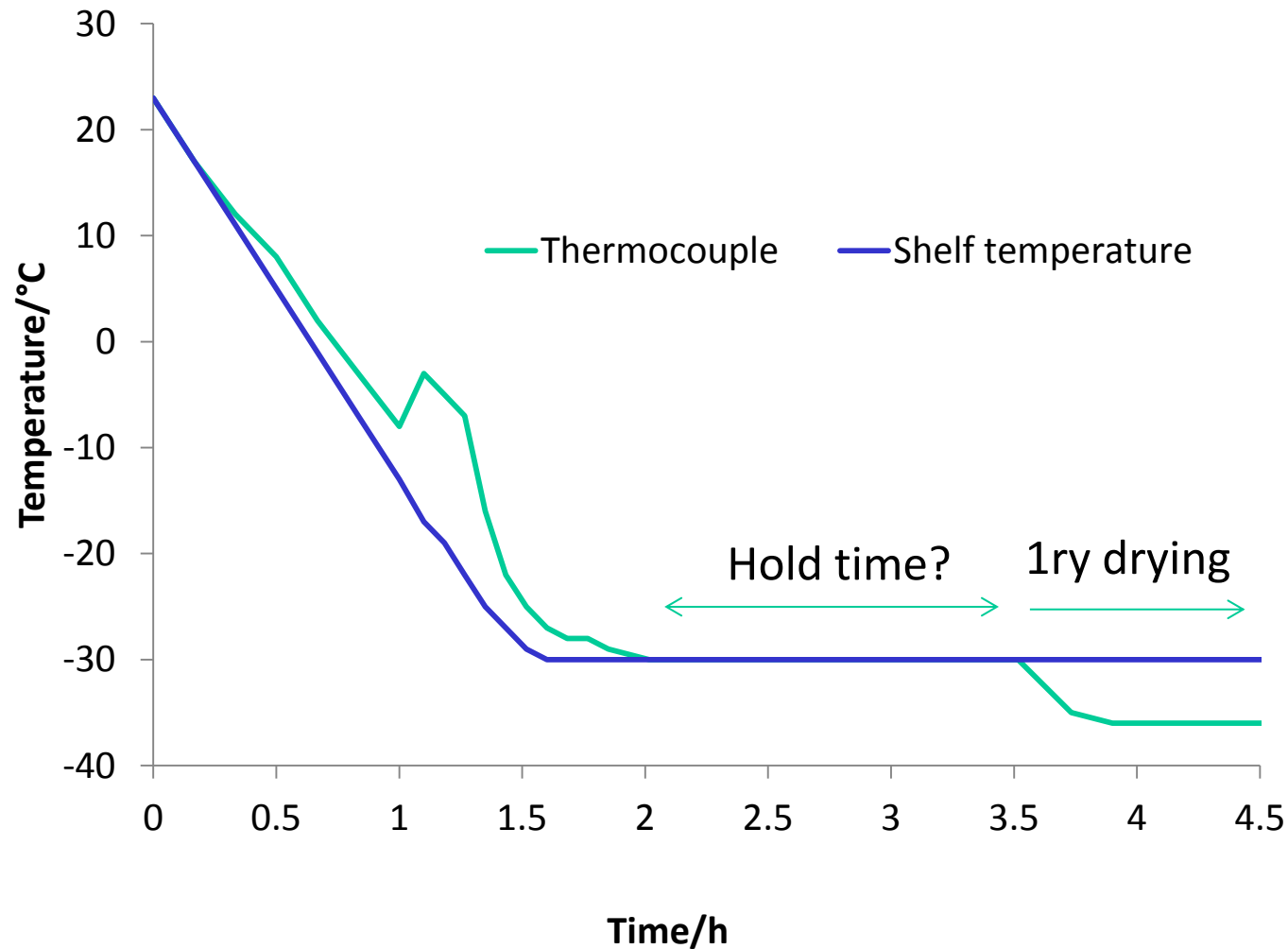


FREEZING STAGE

The Desired state?

(Freezing onset, freezing rate, amount of ice formation,
solidification end point)

End of freezing stage : Product temperature stabilises



Freezing is a critical step

Super-cooling & nucleation, induction

- Ice nucleation is a random process- can impact homogeneity of product
- **Slower freezing** gives rise to bigger ice crystals and permits faster sublimation JA Searles et al *J Pharm Sci* 90; 860-71 (2001)
- **Rapid freezing** may be needed for labile products (Åkerblom et al *Infusions Therapie* 1992; 19:283-287)
- **Annealing** (raising the temperature during freezing stage) may improve ice crystal growth JA Searles et al *J Pharm Sci* 90;872-87 (2001)



Impact of formulation on critical temperatures

When freezing : Ice formation followed by **crystallization of excipients&drug** and/or **formation of the amorphous state**

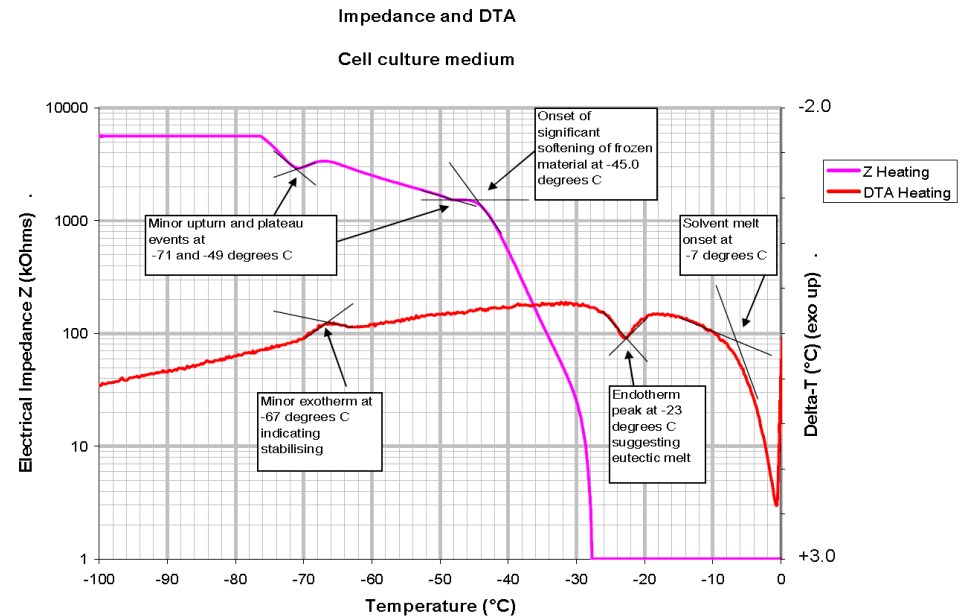
- Characteristics of excipient may define whether it is a crystallising excipient (mannitol) or a glass forming excipient (sucrose)
- Freeze to well below the critical temperatures (eutectic) and hold to ensure complete solidification **(But for how long?)**
- Formulation changes (e.g. mixtures) may result in marked changes in critical temperature **(But are off-line measurements representative?)**
- Crystallisable excipients may require annealing **(But at what temperature and for how long?)**



PAT for laboratory studies : Critical temperatures T_m , T_e , T_g

Lyotherm2 – integrated electrical Impedance ($Z\sin\phi$) and DTA

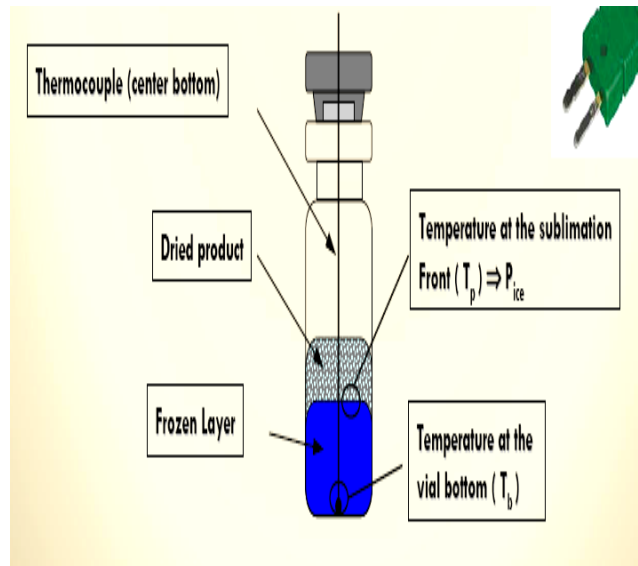
designed to measure glass transition (T_g'), eutectic (T_e) and melting (T_m) temperatures relevant to freeze-drying formulations



Ward & Matejtschuk , 2010 in *Freeze Drying/ Lyophilization of Pharmaceutical & Biological Products* 3rd ed. Rey,L & May JC eds, Informa Press, New York



PAT in freezing stage limited to Temperature Measurement



Thermocouple

- positioned *bottom-centre in the vial*
- less robust (*difficult to handle, sterility problems*)
- Used mainly in *laboratory scale*



1PT100G RTD Elements

Resistance thermal detectors

- Reliable, easy to sterilize
- accurate positioning is difficult, limited validity due to measurement of temperature average
- Used primarily in manufacturing



LyoDEA Brief Description

- The system connects via a junction box to 5 individual **LyoDEA™** test vials positioned around the freeze-drier shelf.
- Frequency scans (10 Hz – 1 MHz) of the LyoDEA™ test vial impedance were recorded every 1-5 minutes throughout a freeze-drying cycle (20 s for each spectrum)
- The **LyoDEA™** measurement and control software saves the spectra from each time point



**LyoDEA
Measurement
Vial**

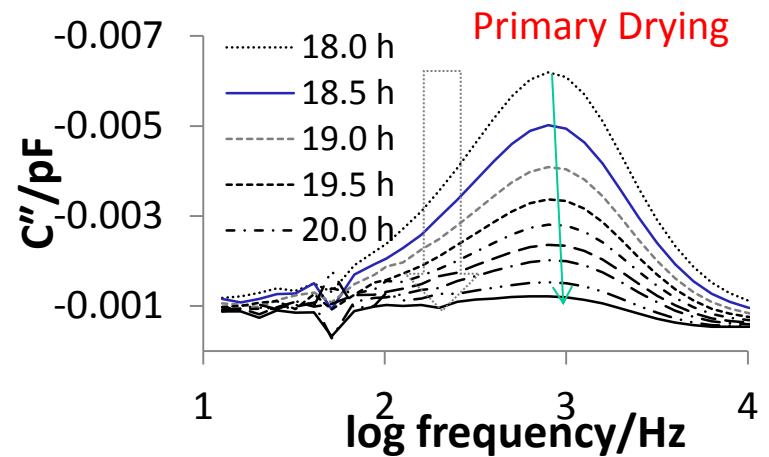
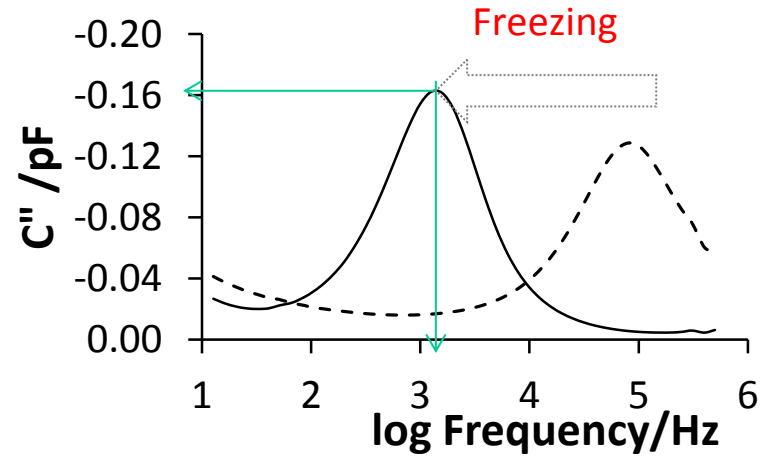
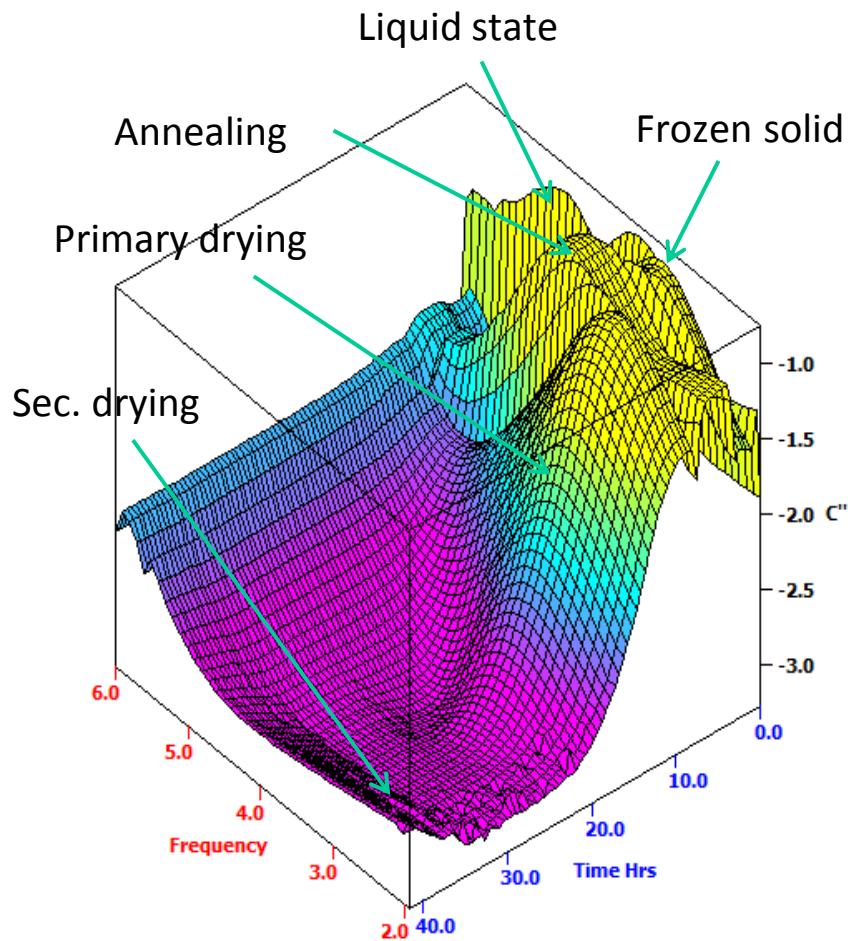


Applications

- Formulation variables
- Freezing rates/end points
- Eutectic crystallization
- Glass transition
- Structural relaxation
- Process variables
- Temperature
- Annealing – ice growth rates
- Drying rates
- Primary drying end points

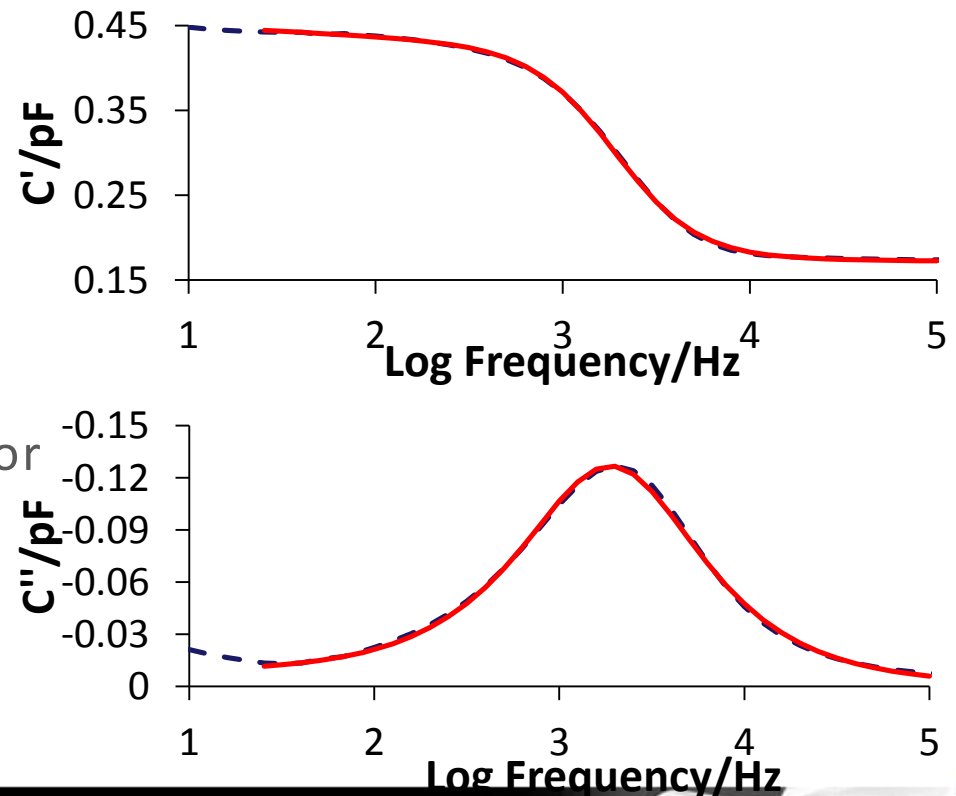
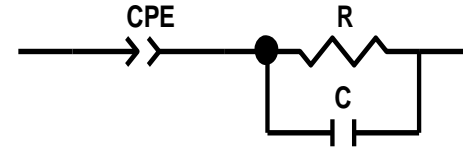


LyoDEA response surface



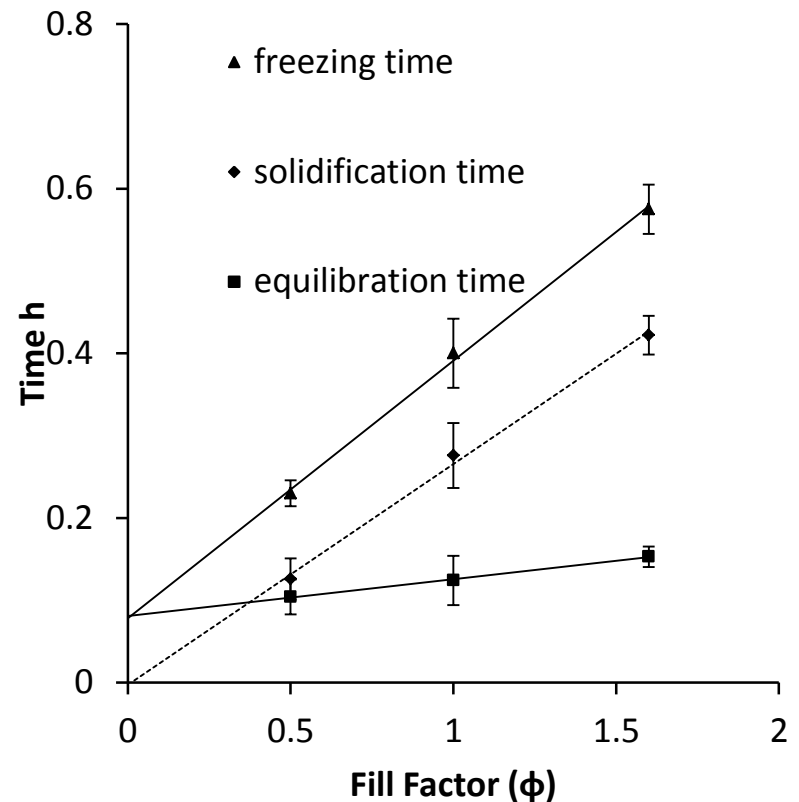
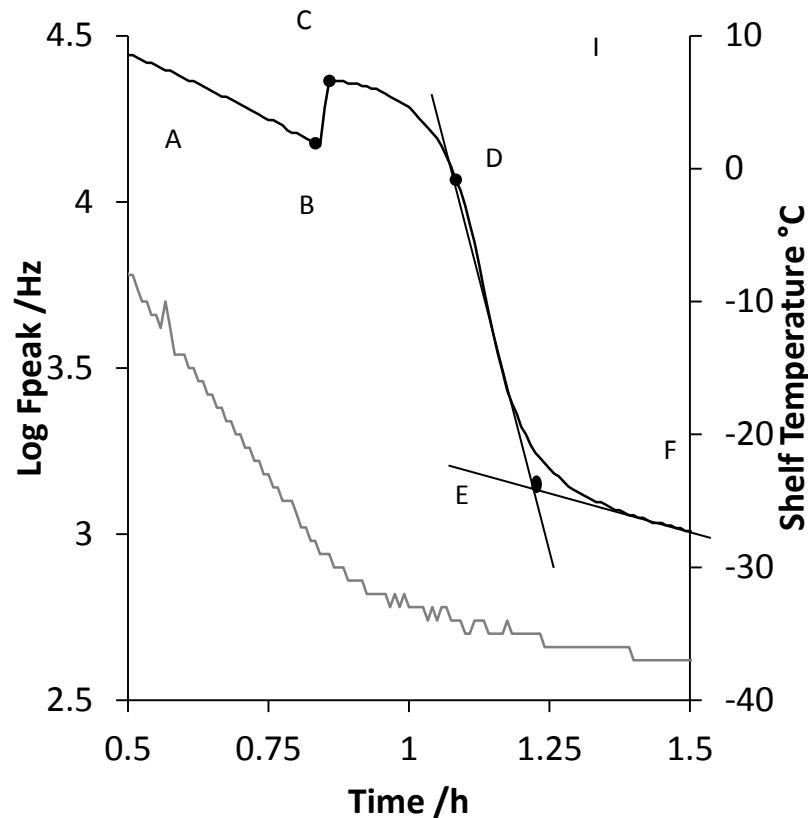
Impedance Modelling

- CPE explains the interfacial impedance of the glass wall of the vial.
- Resistance element records conductivity of ions
- Capacitor element defines dielectric properties of the product.
- The circuit element R was shown to be a sensitive indicator of the phase behavior of the solution, i.e. ice formation and solute crystallization during the freezing cycle

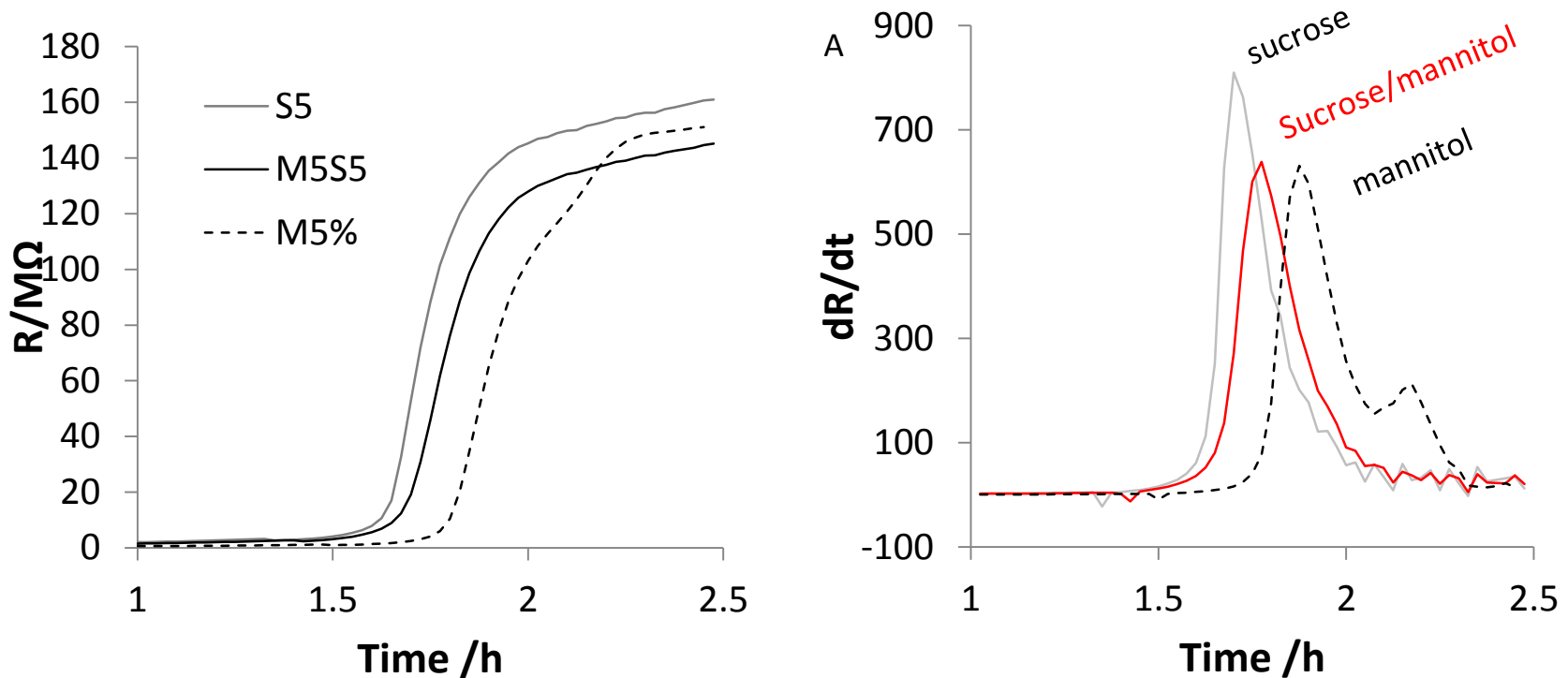


Product Characterization – Ice formation

Fpeak profile records freezing step (B-E) which progress through 2 discrete stages; solidification(B-D) and equilibration(D-E). Time duration for the former increase with the fill height while the latter remain broadly unchanged as it is related to thermal coefficient of the vial base.



Effect of Sucrose on mannitol crystallization

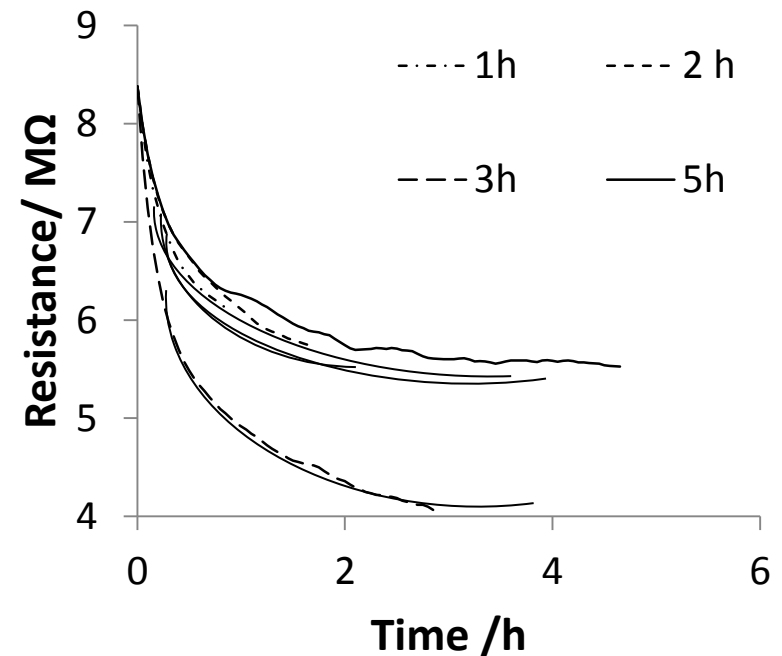
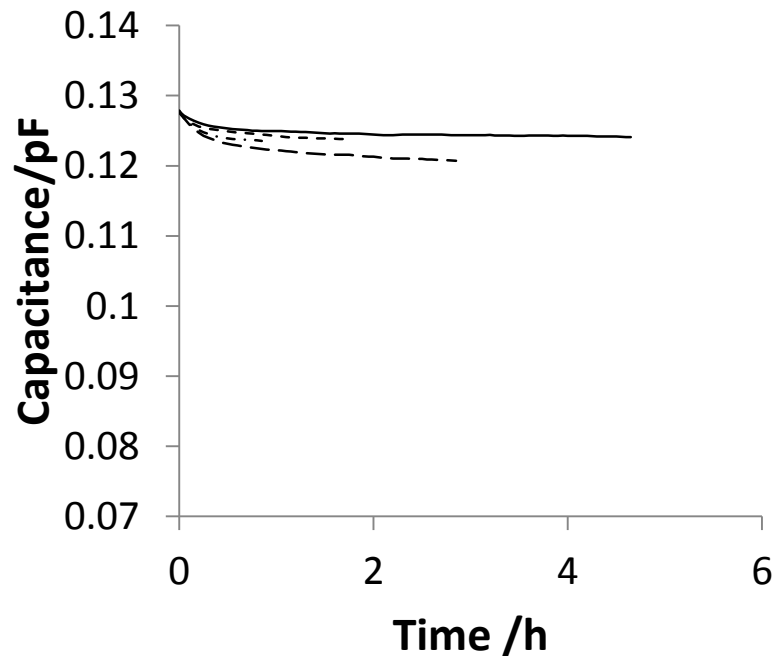


Mannitol crystallization suppressed with the inclusion of sucrose in the solution.



Completion of Annealing (Maltodextrin 10% w/v)

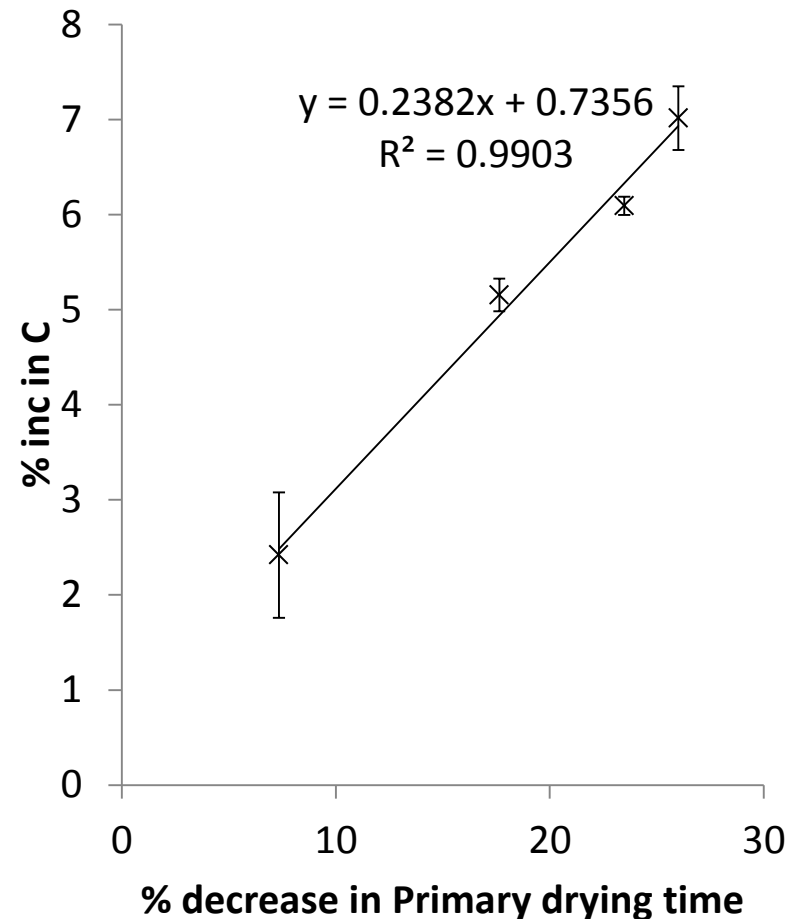
- The capacitance of the formulation changes minimally while the resistance changes significantly and plateaus at 3-4 h
- After 3h annealing hold time, both the capacitance and drying time changes insignificantly .



Predictive control of the primary drying time

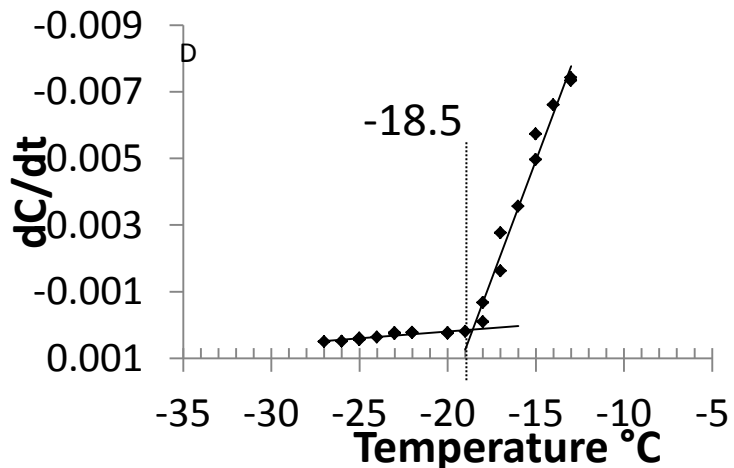
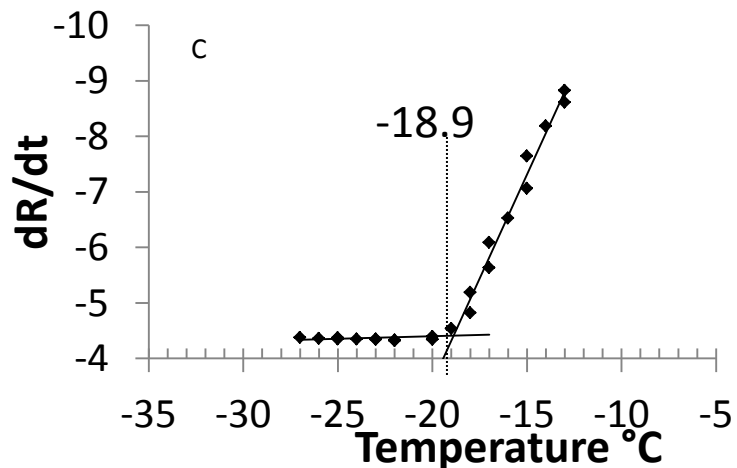
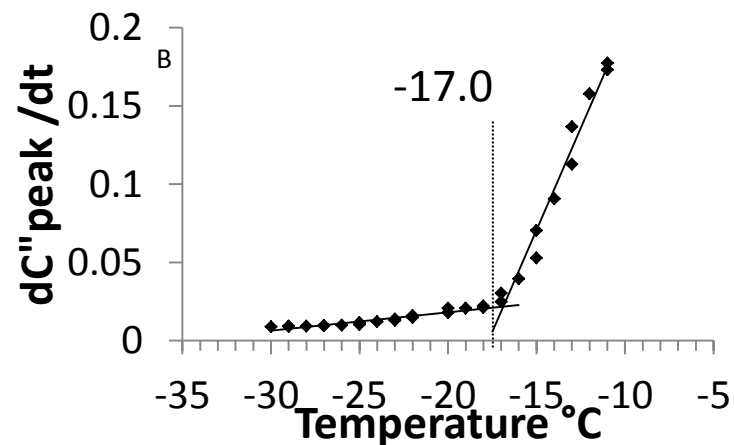
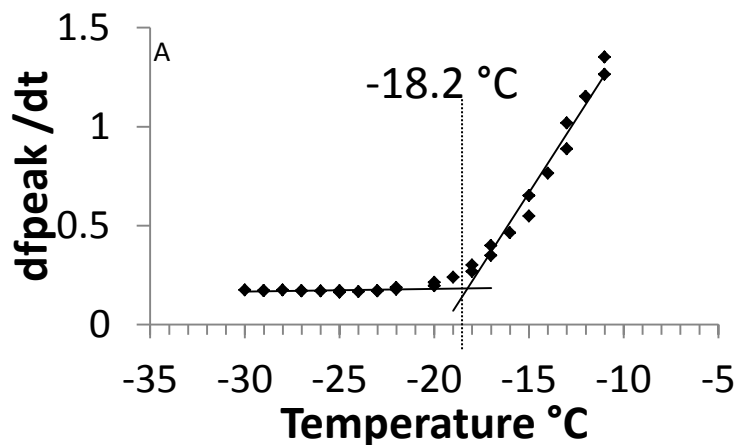
- Increase in the capacitance correlates well with the decrease in the primary drying time.
- Changes in the Product capacitance during annealing may be predictive of the reduction in primary drying time.

For every ~2% increase in capacitance the primary drying decreases by ~8%



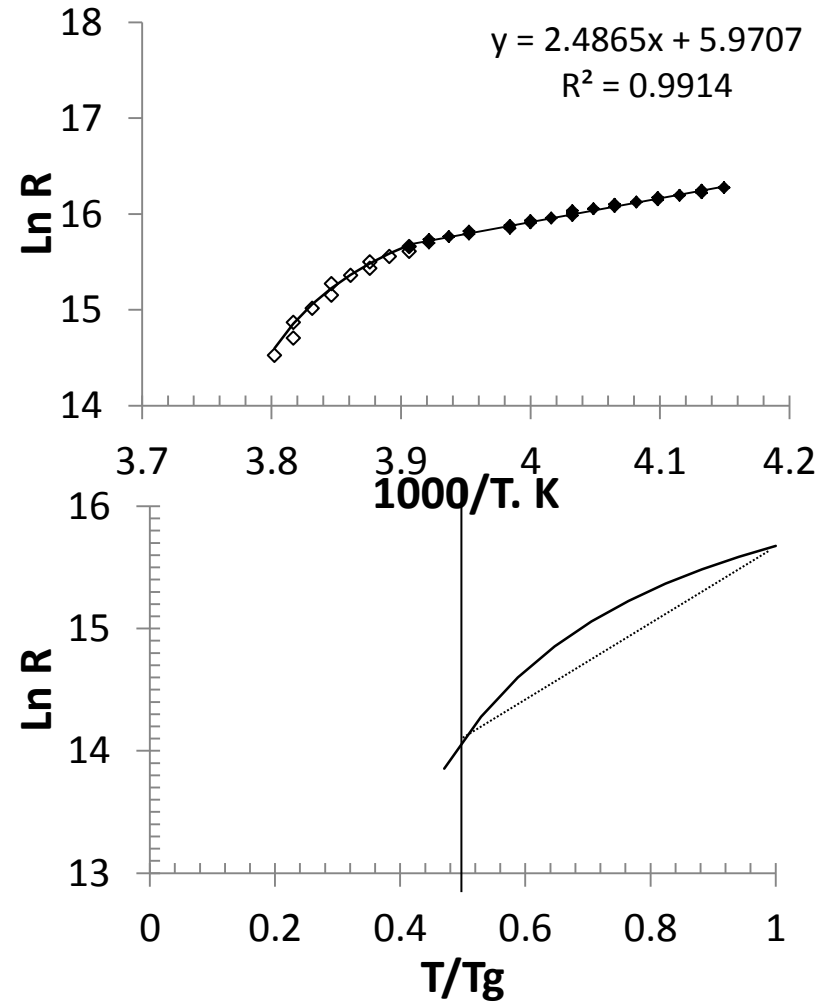
Product Characterization: T_g determination by LyoDEA

Aqueous solution of Malto-dextrin 10% w/v by LyoDEA on heating at $1\text{ }^{\circ}\text{C min}^{-1}$



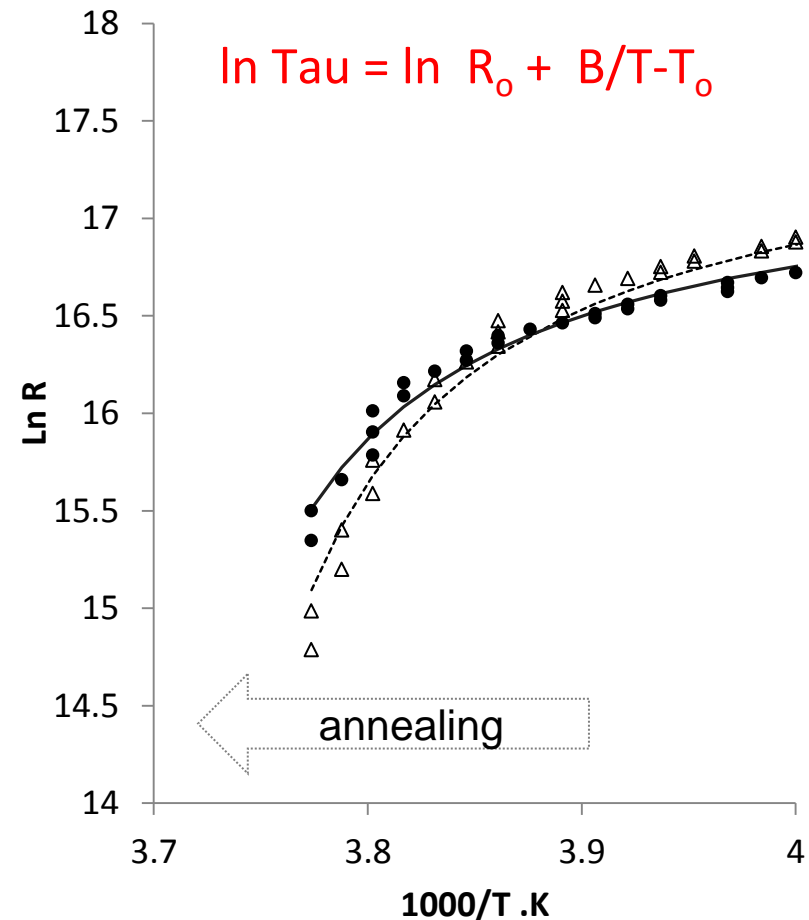
Measurement of Fragility of frozen solution(Maltodextrin 10% w/v)

- Below T_g the changes in product resistance follows Arrhenius trend with $E_a \sim 20 \text{ KJ.Mol}^{-1}$.
- Above T_g VTF function models the resistance profile.
- The fragility of the glassy matrix calculated from VTF results and slope of resistance was recorded to be ~ 0.7 ; suggesting a fragile glass.



VTF Fit to describe the above T_g resistance

- Above T_g the temperature dependence of the product resistance follows the Vogel-Tammann-Fulcher function.
- The curvature of the resistance plot decreases following annealing
- which relates to the **increased strength of the glassy material**.



Fragility of different solutions

Solution details	Fragility*
Maltodextrin 10% w/v	0.7
Lysozyme 4.5% w/v	0.8
Lysozyme 4.5 % + trehalose 1.5% w/v	0.6

- The higher the fragility number the more fragile the glass
 - A finger print for stability – reproducibility?

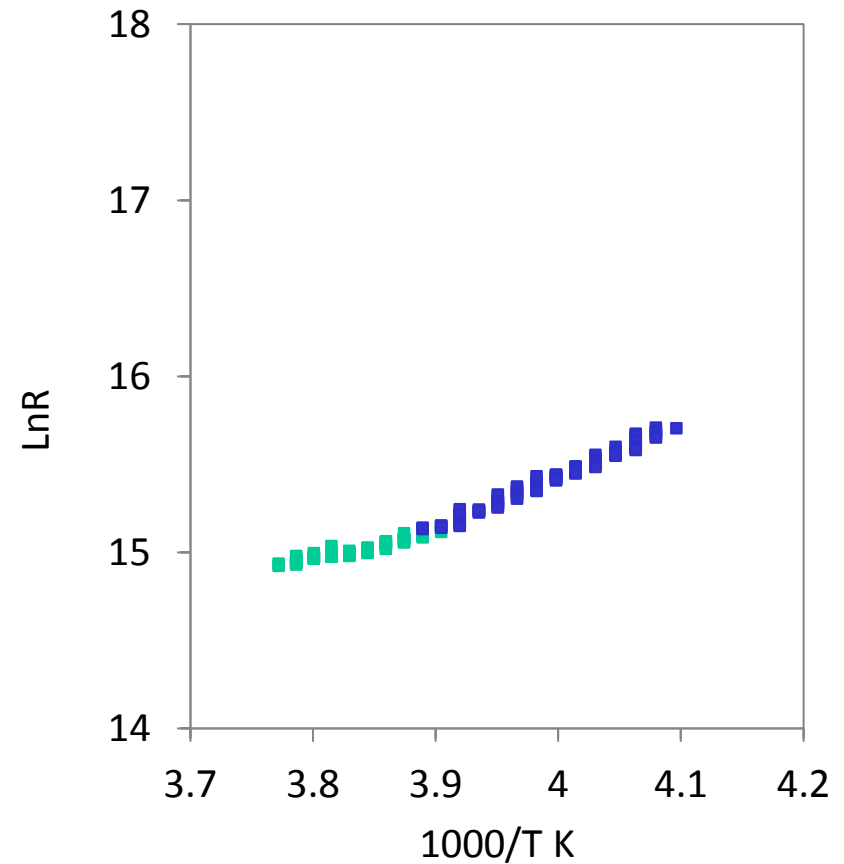
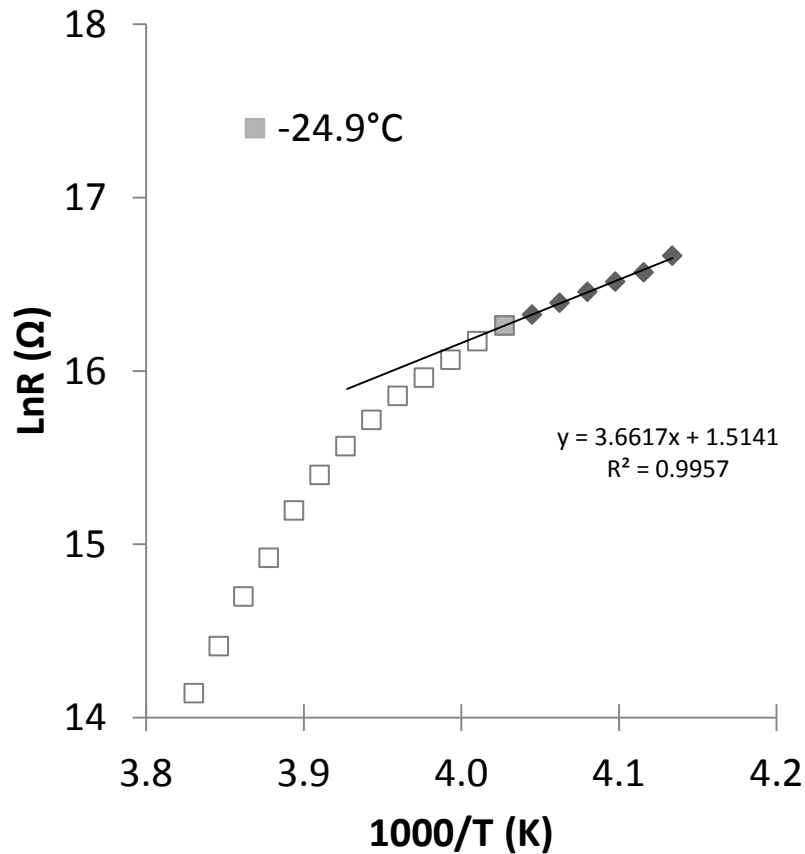
Precision of these numbers in relation to relevance requires validation



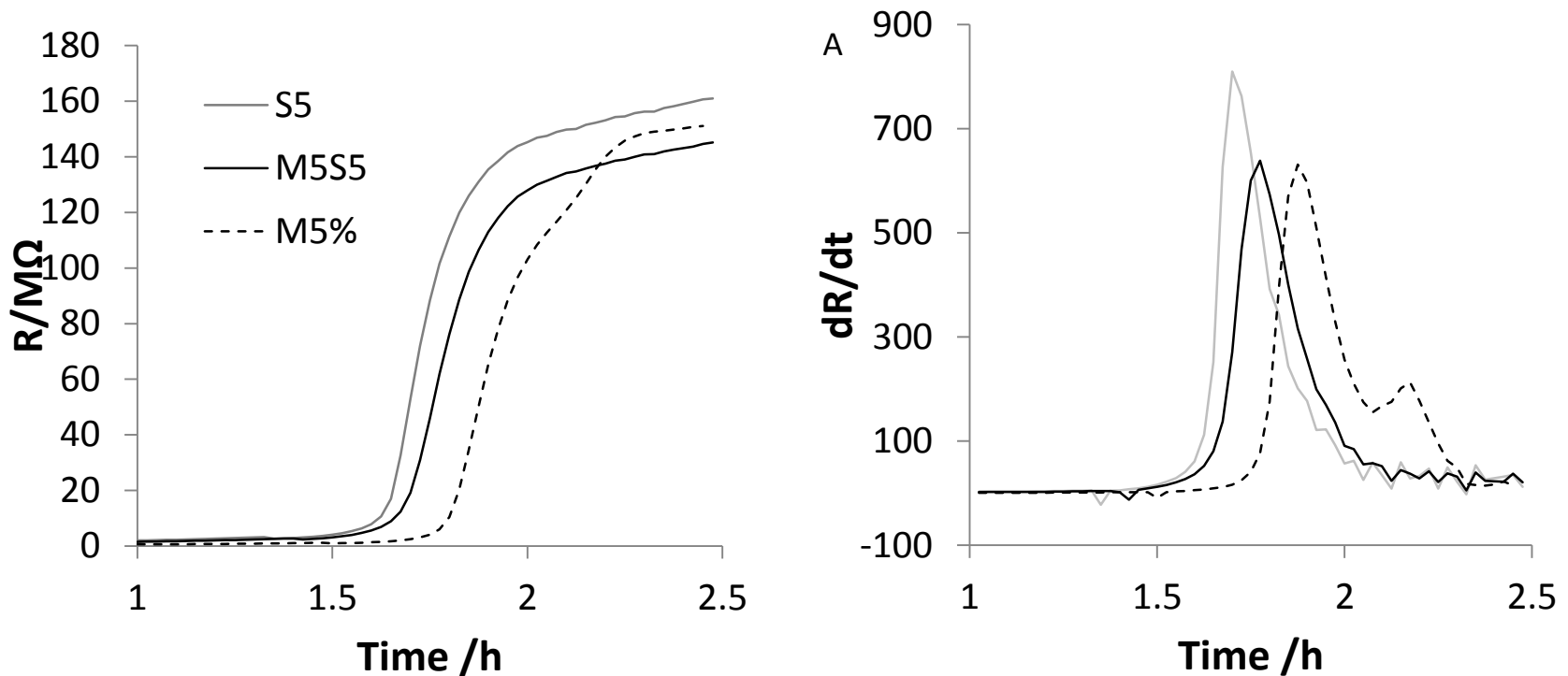
Pre-heated Lysozyme 4.5% w/v (pre-aggregated)

- Lysozyme solution

- Pre-heated Lysozyme solution



Effect of Sucrose on mannitol crystallization



Mannitol crystallization suppressed with the inclusion of sucrose in the solution.



PRIMARY DRYING STAGE

The Desired state?

Fast drying rate, without compromising product quality, or
operating at limits of equipment

Design Space for Primary Drying

- The aim is to achieve an acceptable drying rate, without
 - compromising product quality
 - operating the equipment at (or beyond) the limits of its capability
- Lab scale instruments for screening formulations and process conditions to optimise drying profiles **Microbalance**



- PAT and “intelligent” freeze drying software has allowed
 - in process monitoring
 - interactive control of the cycle



PAT in Primary Drying

Methodologies for Production Scale

- pressure rise and MTM (Tang et al *Pharm Res* 2005,22;685-700),
- tunable diode laser absorption spectroscopy (Gieseler et al *J Pharm Sci* 2007,96;1776-93)
- soft sensor probes (Barresi et al *Int J Refrigeration* 2009,32;1003-14)

have enabled critical process parameters (drying rate) to be monitored and used to drive cycle progression and method optimisation.

- Methodologies for Development/lab Scale

near infra-red & Raman spectroscopy (De Beer et al *J Pharm Sci* 2009,98;3430) to model drying within analytical equipment and to assess stabilization (Hedoux et al *J Pharm Sci* 2013,102;2484-94)



Design Space

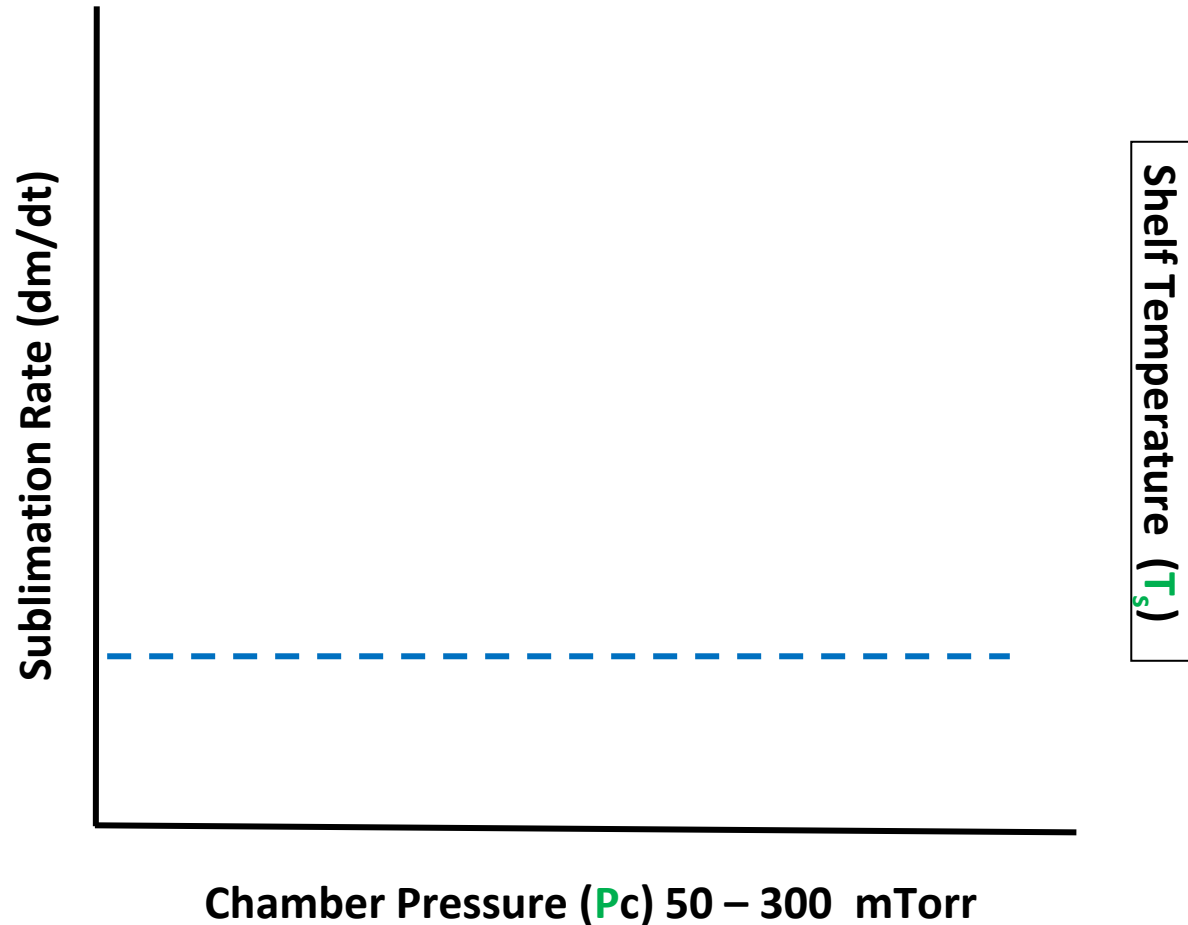
- A target sublimation rate can be achieved by two independently controlled variables:

(i) Chamber pressure (shown on the x axis)

(ii) Shelf temperature (shown as a floating variable)

Chang & Fisher 1995

The dotted line is the minimum acceptable drying rate



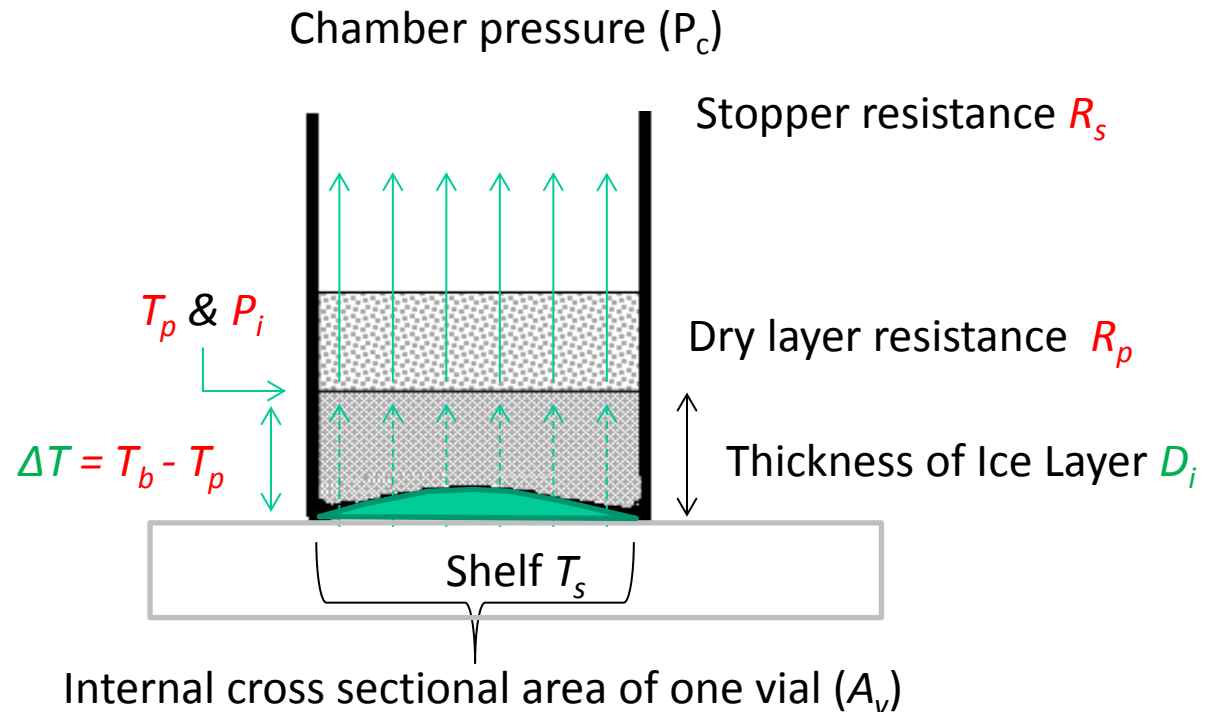
Primary Drying Modelling : Heat and Mass Transfer

$$dm/dt = \frac{A_v(P_i - P_c)}{R_{ps}}$$

$$dq/dt = A_v K_v (T_s - T_p)$$

$$dq/dt = \Delta H_s dm/dt$$

$$\ln P_i = -145/T_p + 24.02$$



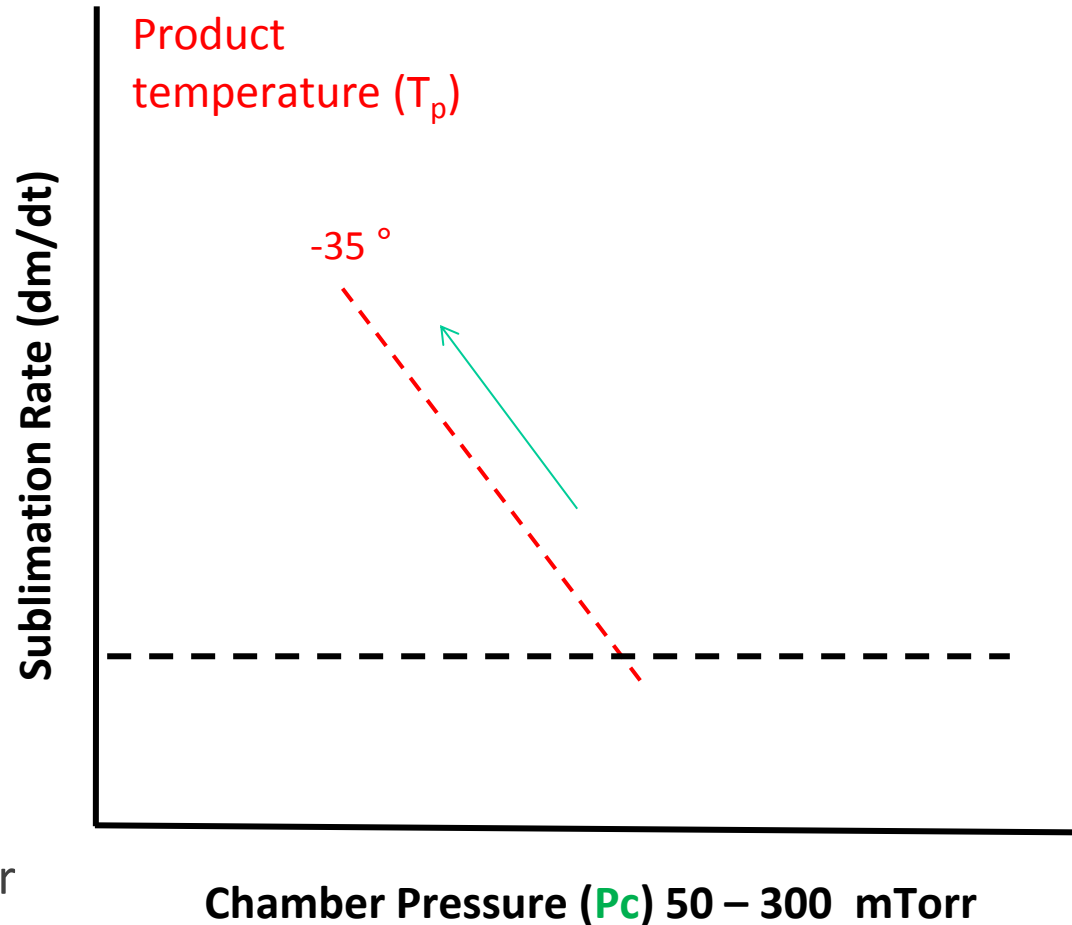
Design Space

$$dm/dt \propto (P_i - P_c)$$

- **Lower chamber pressures (P_c)** increases the driving force for sublimation

$$dm/dt \propto (P_i - P_c)$$

- Effect seen for a constant ice vapour pressure, P_i
- i.e. A constant product temperature (T_p)
- Linear increase in rate with decreasing chamber pressure



Design Space

$$dq/dt = A_v K_A (T_s - T_p)$$

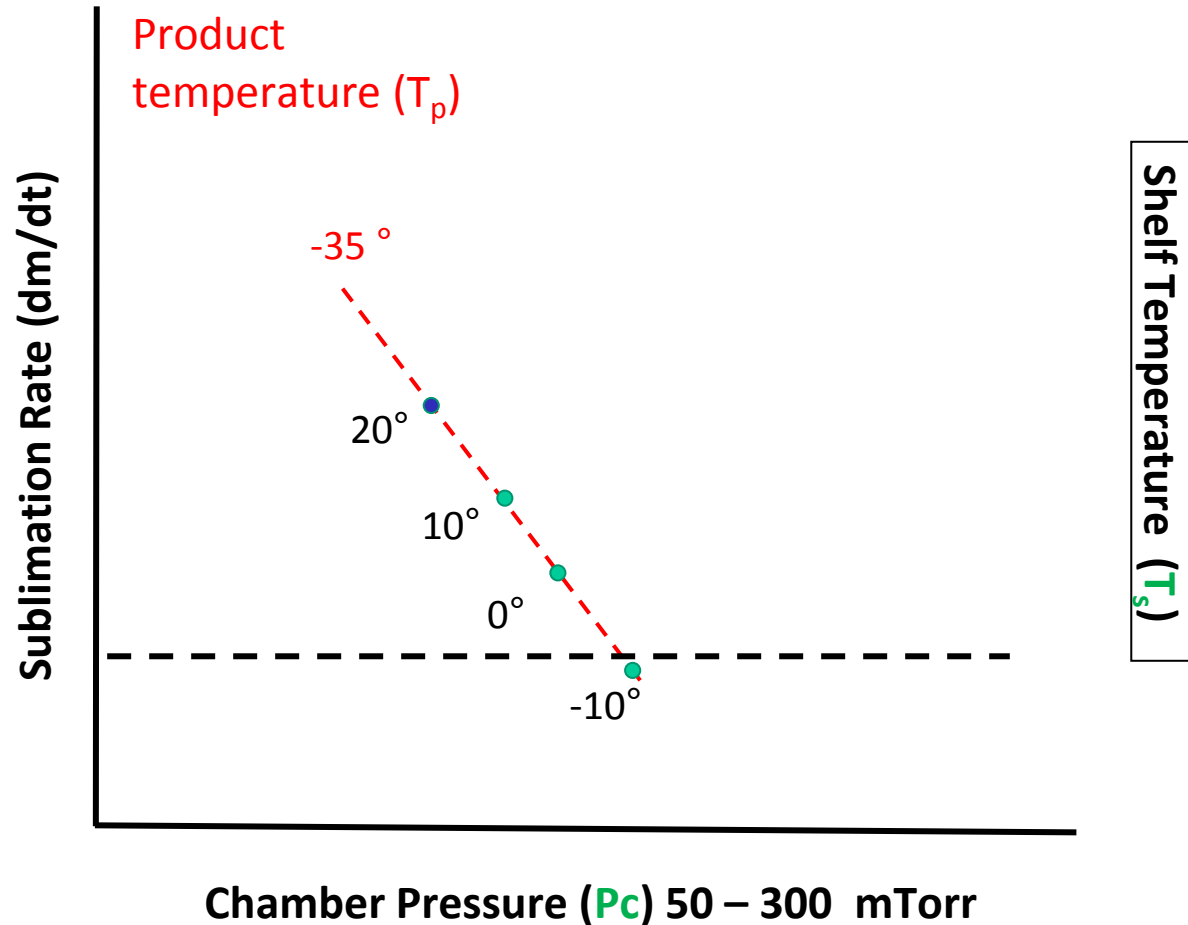
$$dm/dt = -\Delta H_i dq/dt$$

- Increases sublimation rates requires greater rate of heating (dq/dt),

$$dm/dt = -\Delta H_i dq/dt$$

- which, for a constant product temperature, can only come from increasing the shelf temperature (T_s)

$$dq/dt = A_v K_v (T_s - T_p)$$



Design Space

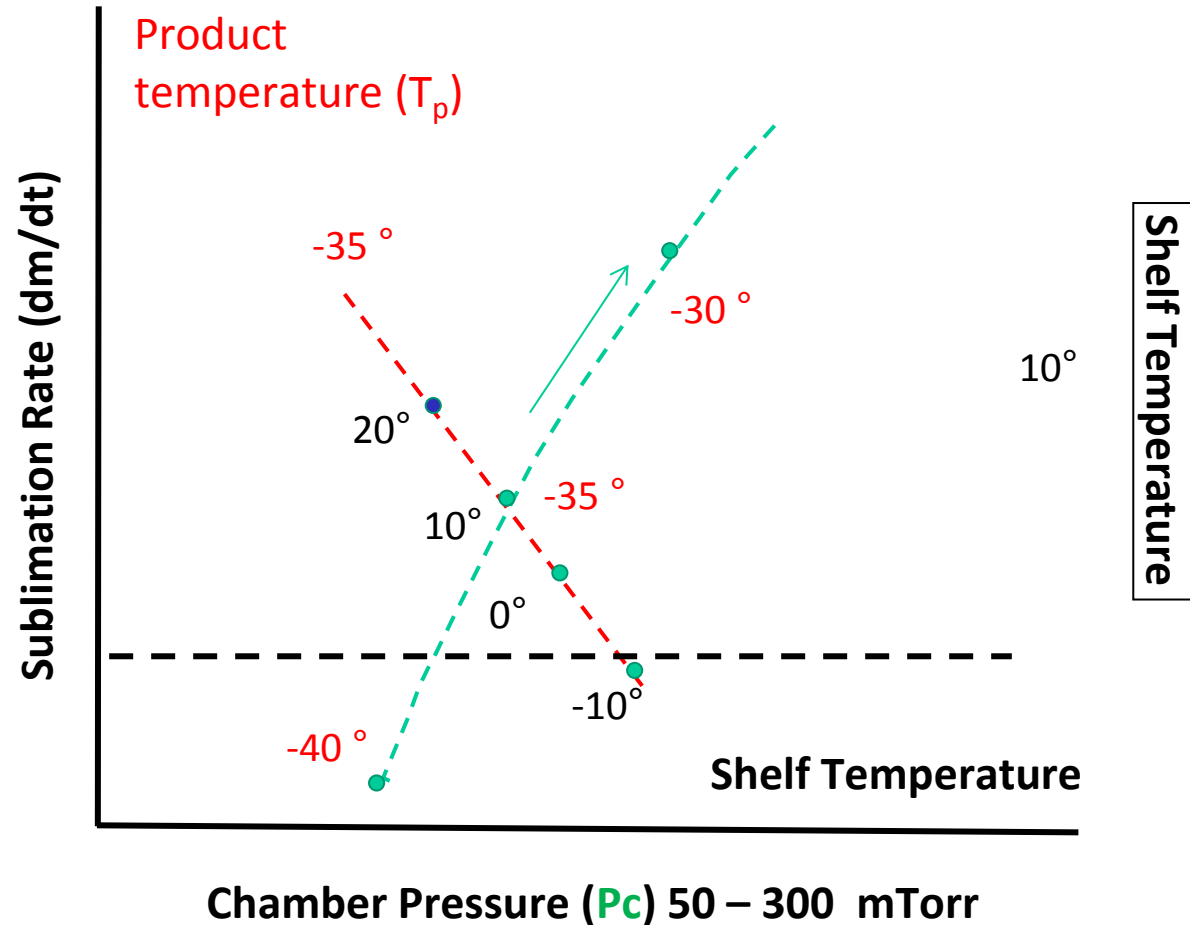
$$dq/dt \propto K_v K_G$$

$$T_p \propto dq/dt$$

$$P_i \propto T_p$$

$$dm/dt \propto (P_i - P_c)$$

- **Higher chamber pressures** also increases rate of heat transfer by increasing the thermal conductance of the gas in the gap between the shelf and the bottom of the vial (K_G)
- This in effect increases the product temperature (T_p) which increases the ice vapor pressure, increasing driving force for flow of vapour in the chamber



Design Space

$$dq/dt \propto K_v K_A$$

$$T_p \propto dq/dt$$

$$P_i \propto T_p$$

$$dm/dt \propto (P_i - P_c)$$

Two conflicting driving forces associated with increased chamber pressure

$$dm/dt \propto (P_i - P_c)$$

AND

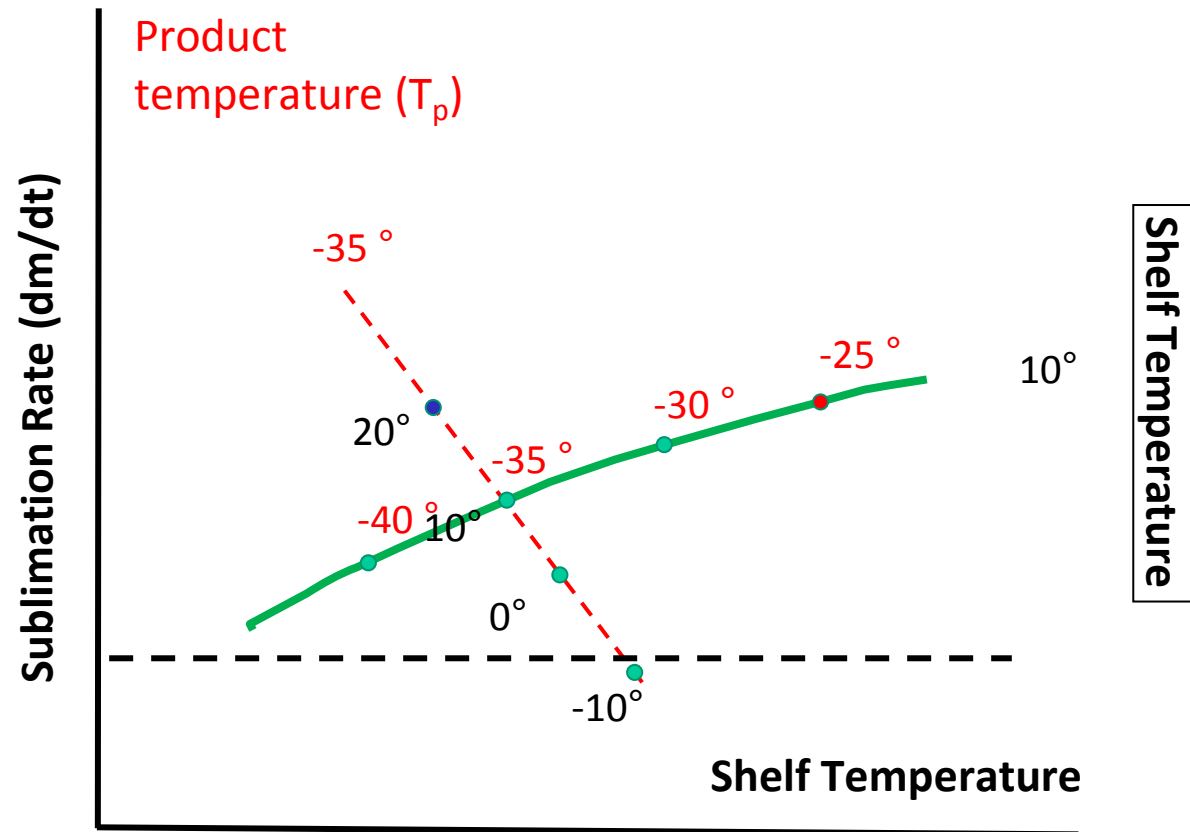
$$dm/dt \propto (P_i - P_c)$$

THE RESULT

$$dm/dt \propto P_i$$

THE LESSON

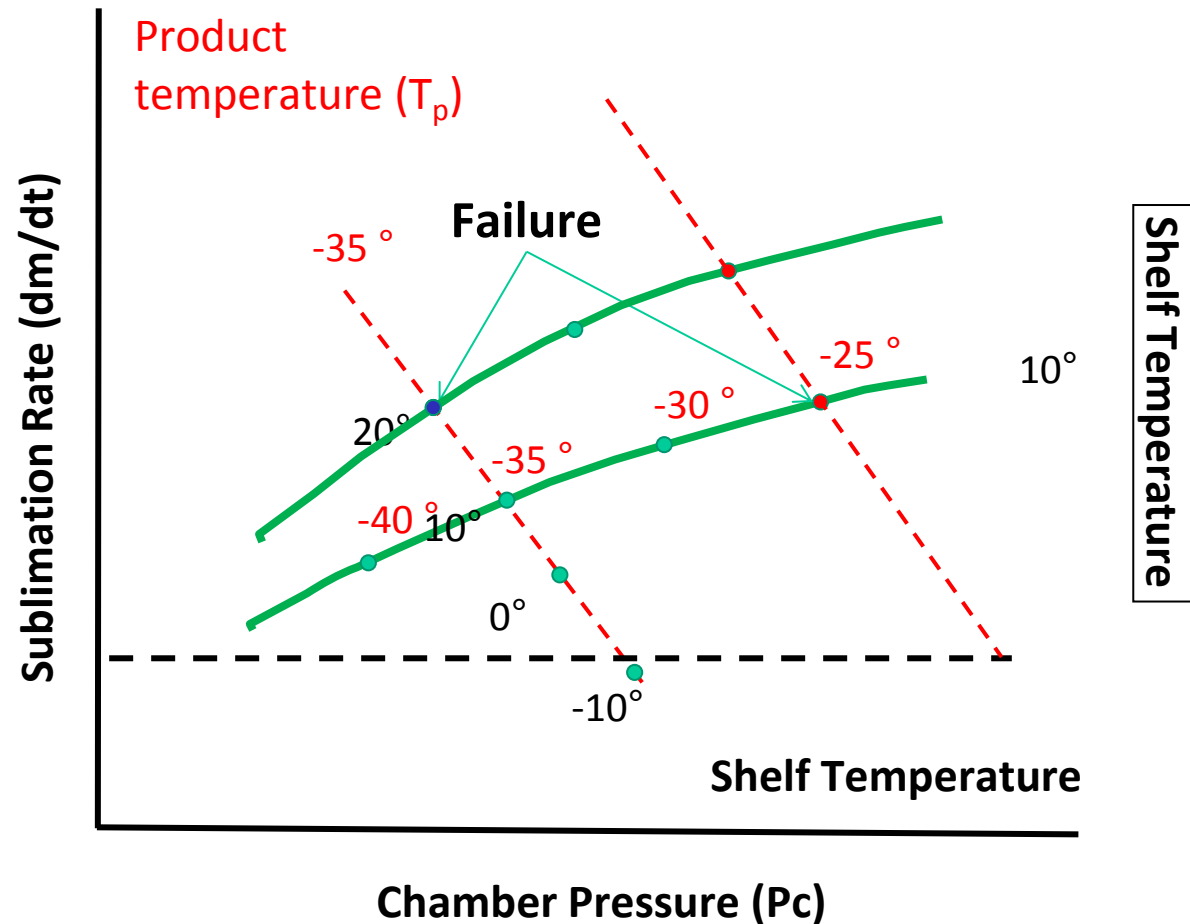
Drive process via T_p



Design Space – Failure Modes

There are two failure points resulting from

- (i) Formulation
- (ii) Equipment



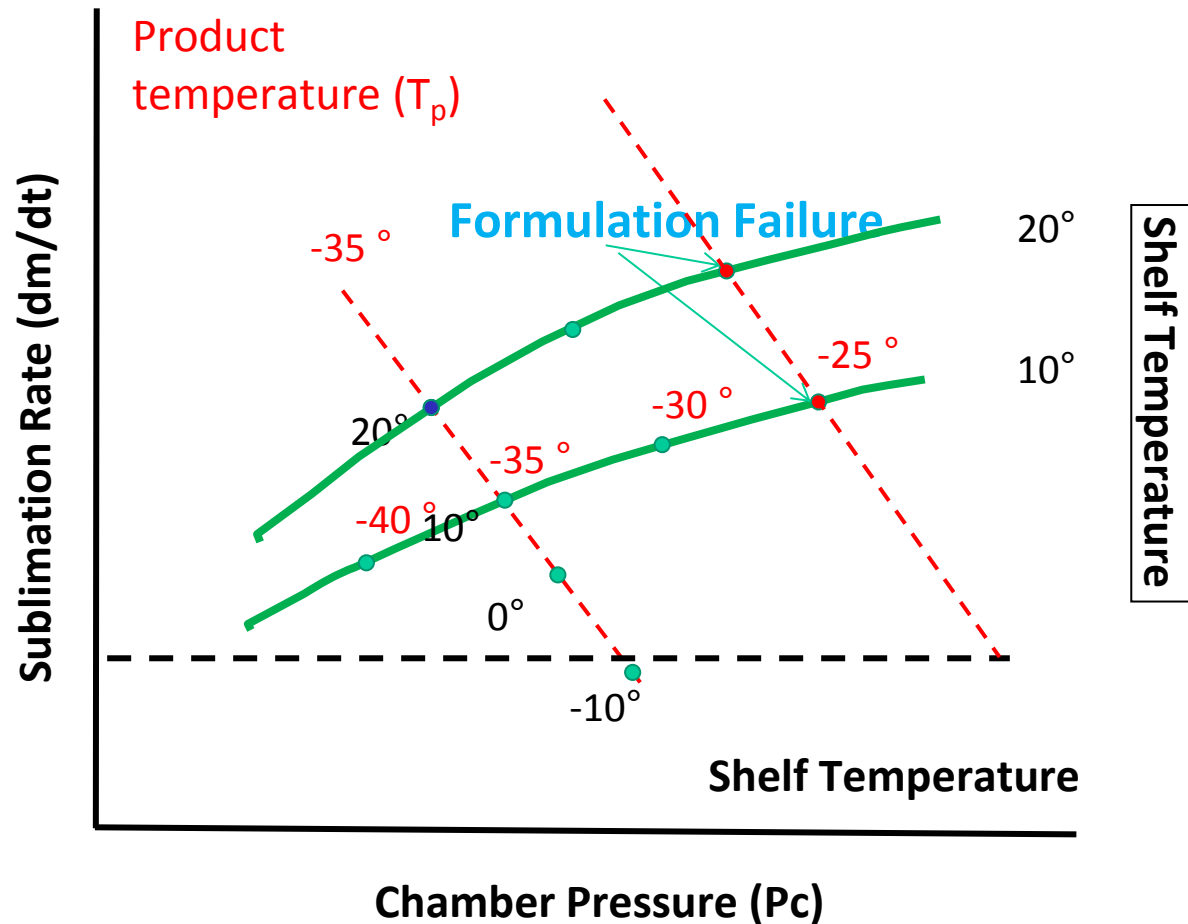
Design Space

There are two failure points resulting from

- (i) **Formulation**
- (ii) **Equipment**

Formulation Limits

If the product exceeds its critical temperature at which the viscosity of the unfrozen matrix is too low to support its weight then it collapses (e.g. -25 C)



Design Space

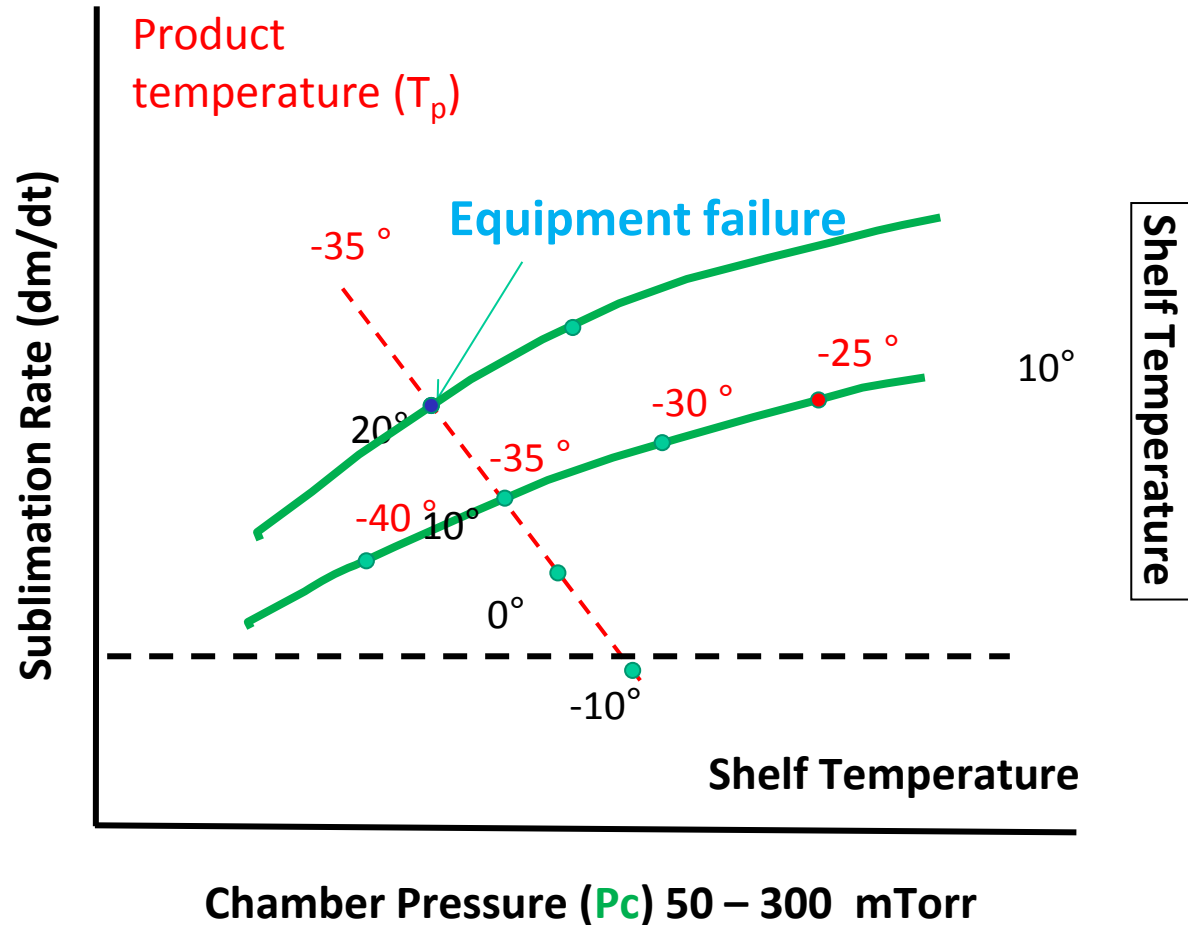
There are two failure points resulting from

- (i) **Formulation**
- (ii) **Equipment**

Equipment Limits

Condenser capacity to trap ice and maintain its temperature

Shelf coolant capacity to maintain its temperature

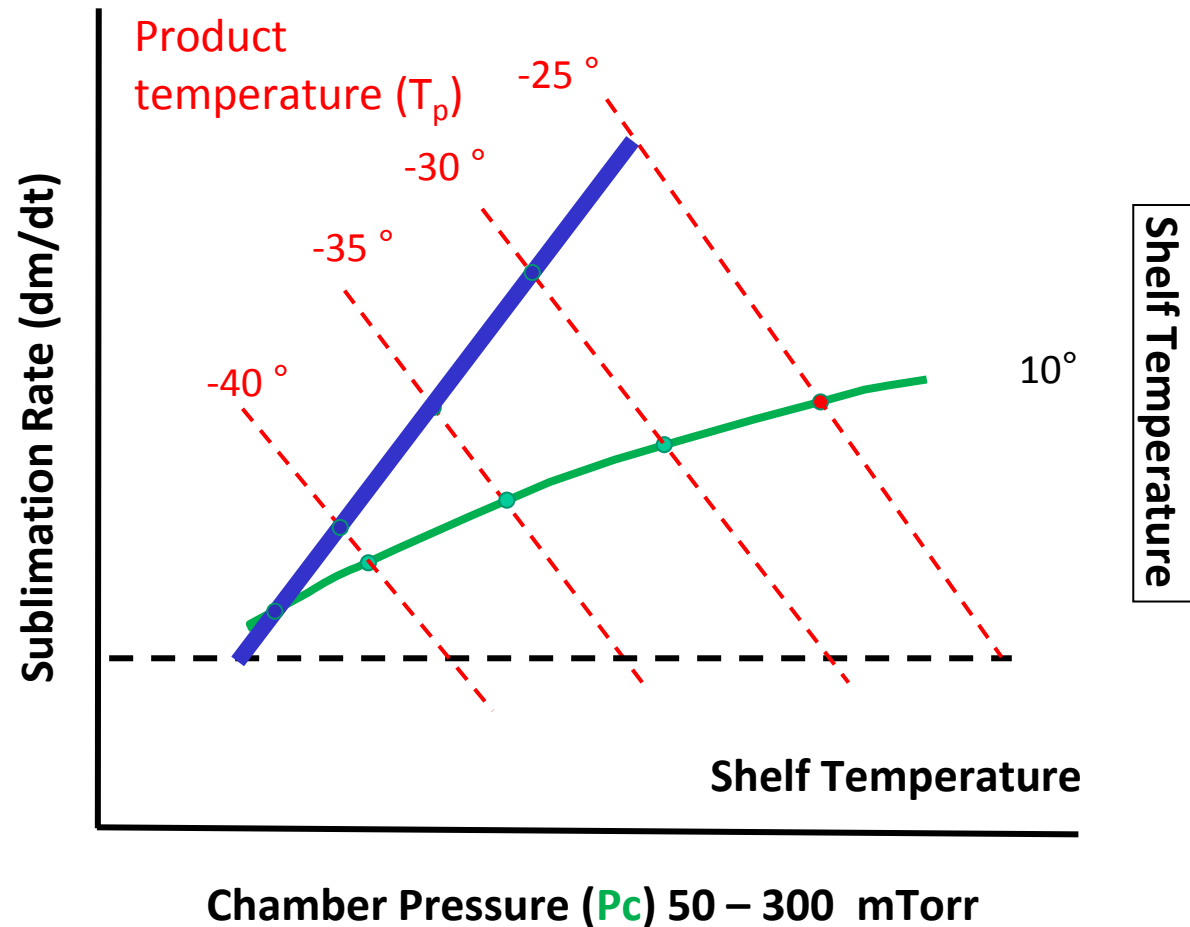


Design Space Choked flow

$$dm/dt \propto (P_i - P_c) = 0$$

With aggressive drying the sublimation rate is eventually suppressed by the maximum volume of water vapour which could traverse from chamber to condenser in unit time

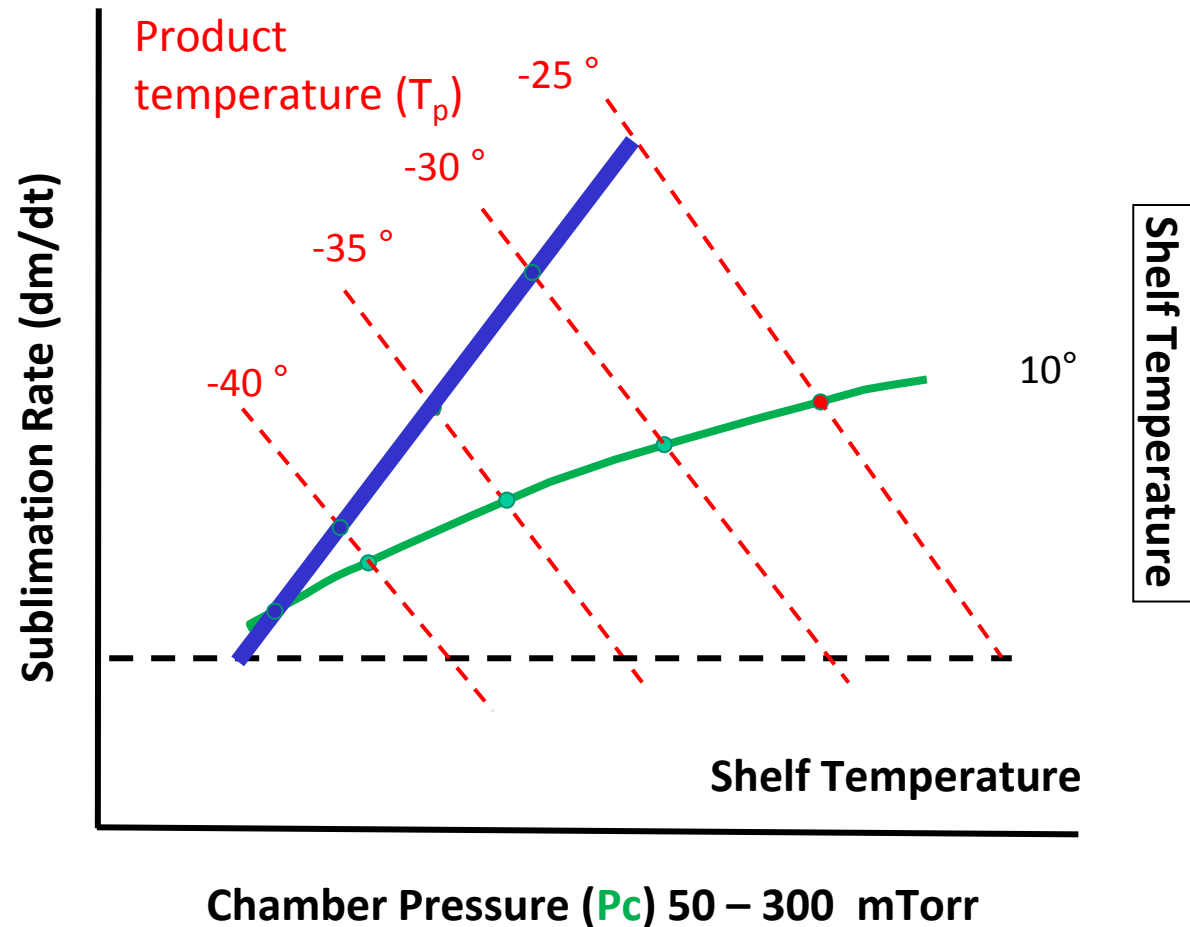
$$P_i \approx P_c$$



Design Space Choked flow

$$dm/dt \propto (P_i - P_c) = 0$$

Similar effects have been observed with the physical spacing of the shelves in a stack can also pose a resistance to the increasing sublimative flow, with pockets of greater chamber pressure building up between narrowly separated shelves and limiting the effective drying rate.



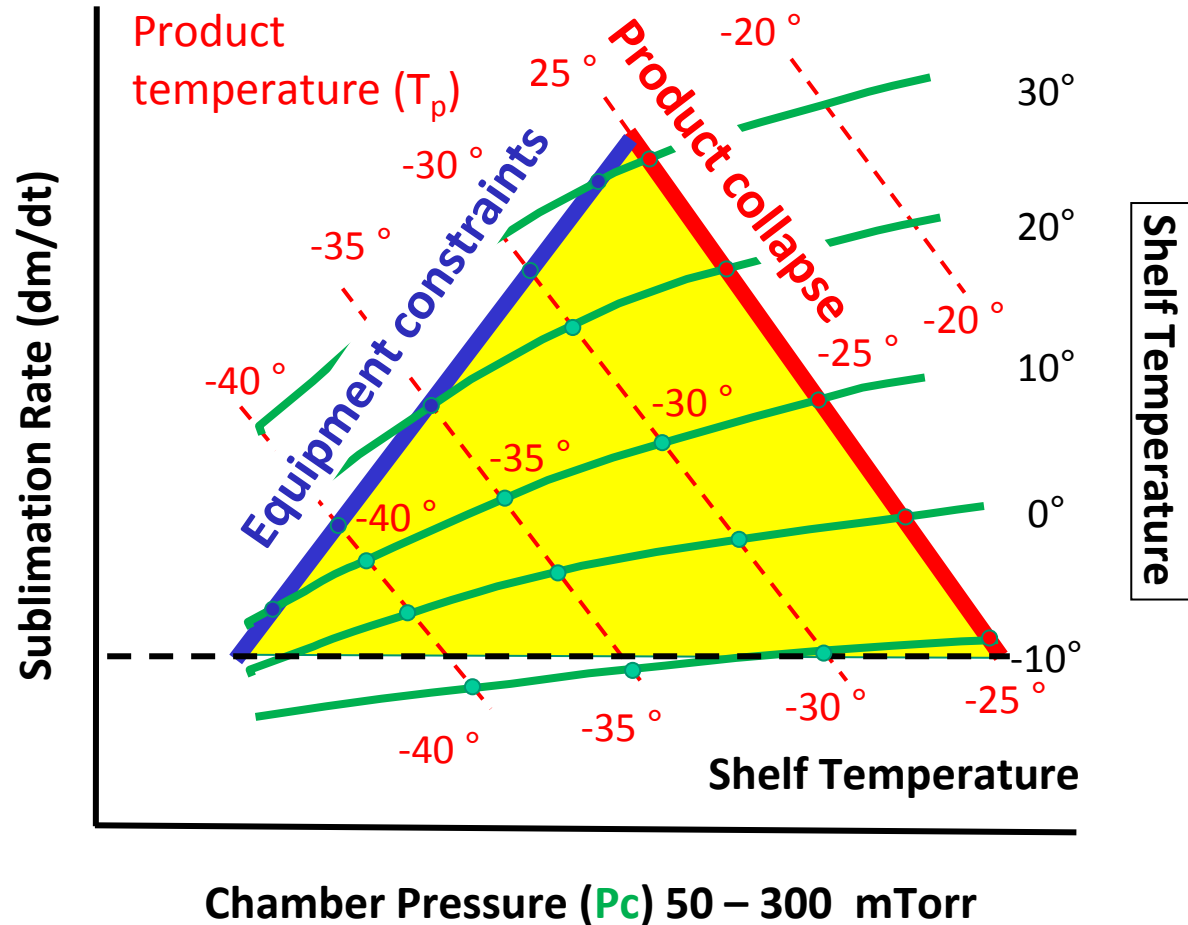
Design Space

Complete the DoE!!

Using a range of chamber pressures and shelf temperatures to establish the limits of the equipment

The design space is shown by the yellow triangle

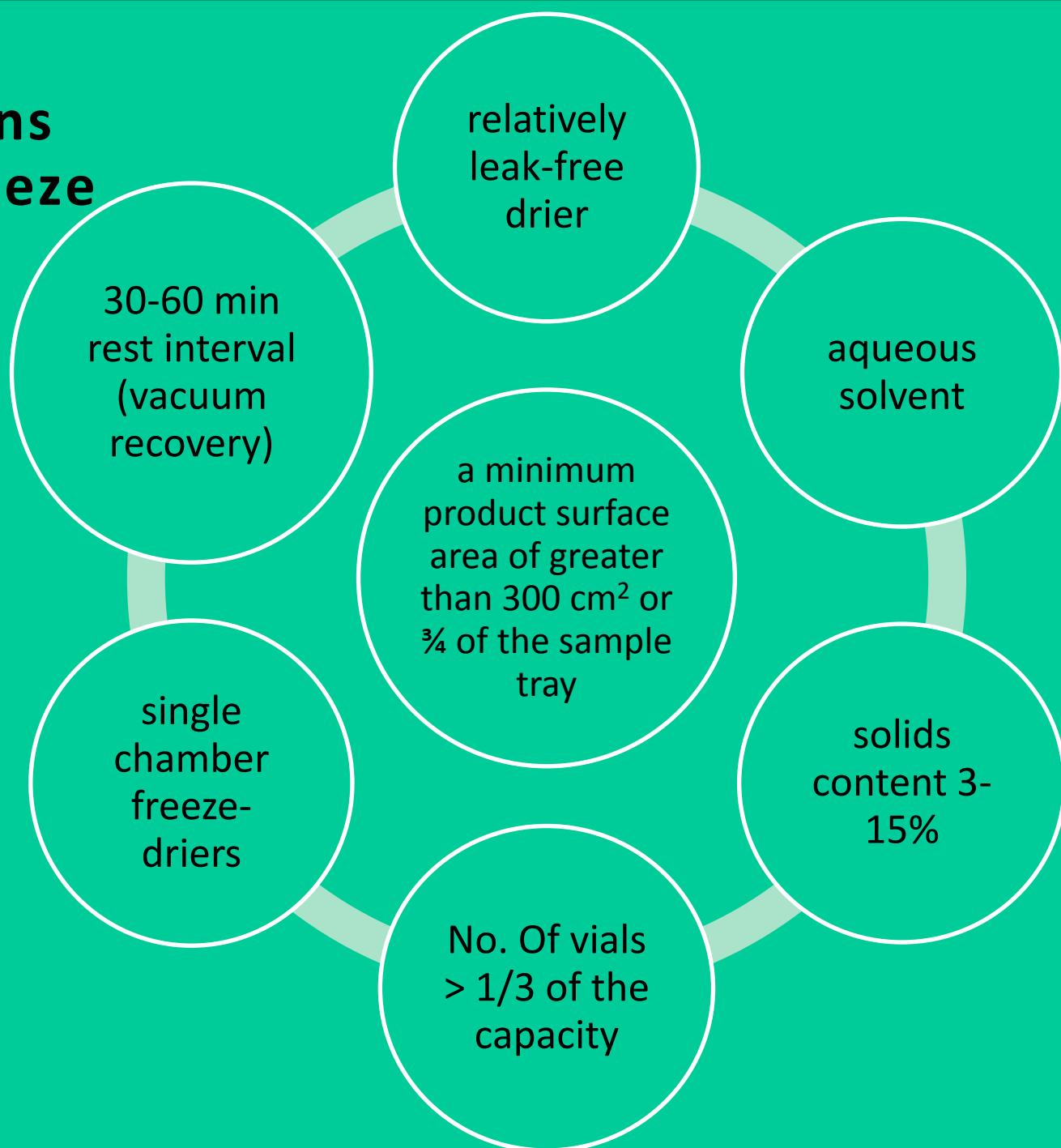
Operate at the apex of the triangle to drive process efficiencies



$$D_i(t) = \frac{m(0) - m(t)}{q_i A_p \mathcal{E}}$$

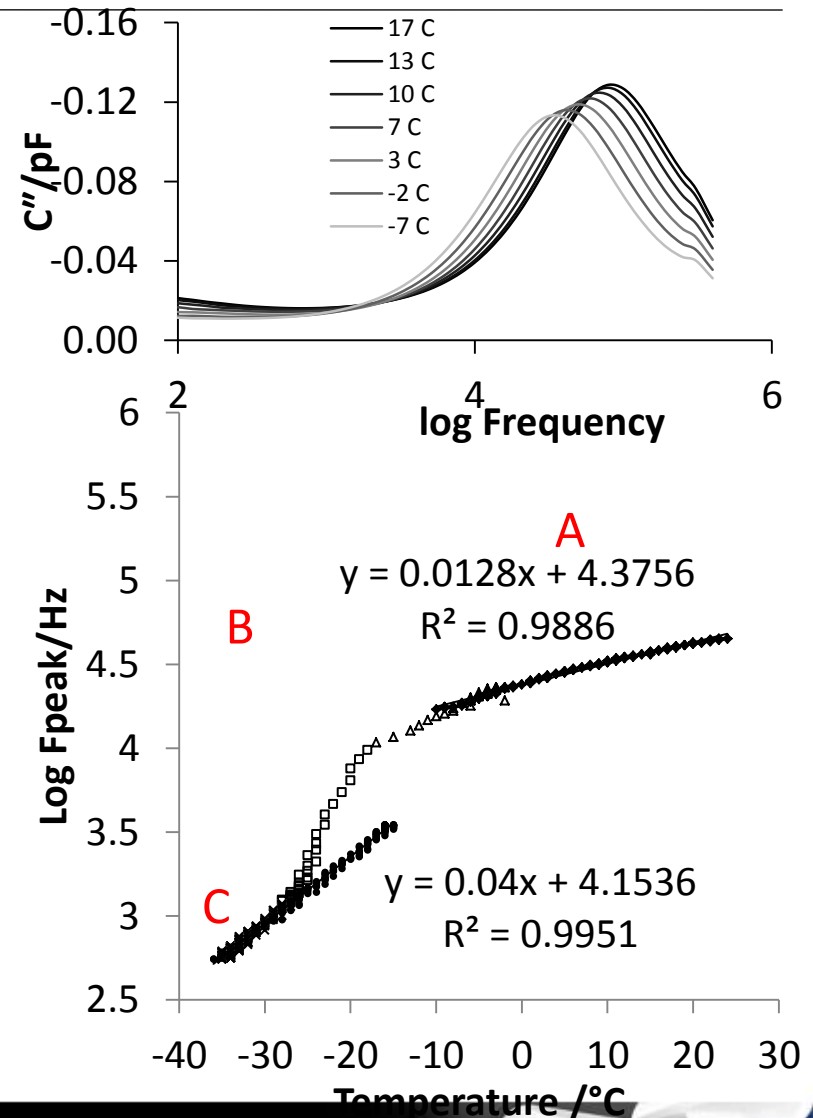


Limitations Smart freeze drying



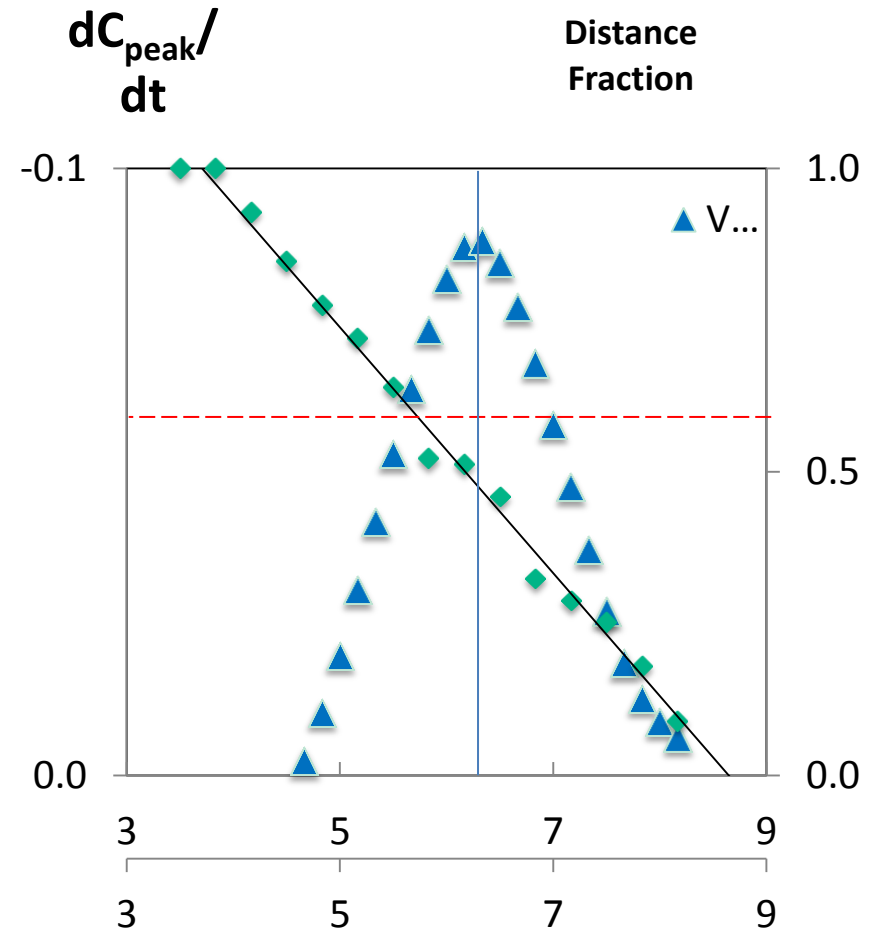
Product Characterization : phase behaviour, temperature

- The f_{peak} showed a good correlation with the product temperature during product cooling (A), freezing (B) and thawing (C)
- Provided there is no change in phase, then a linear correlation exists between Log F and temperature (A, C-D)
- Use LyoDEA response to drive the process



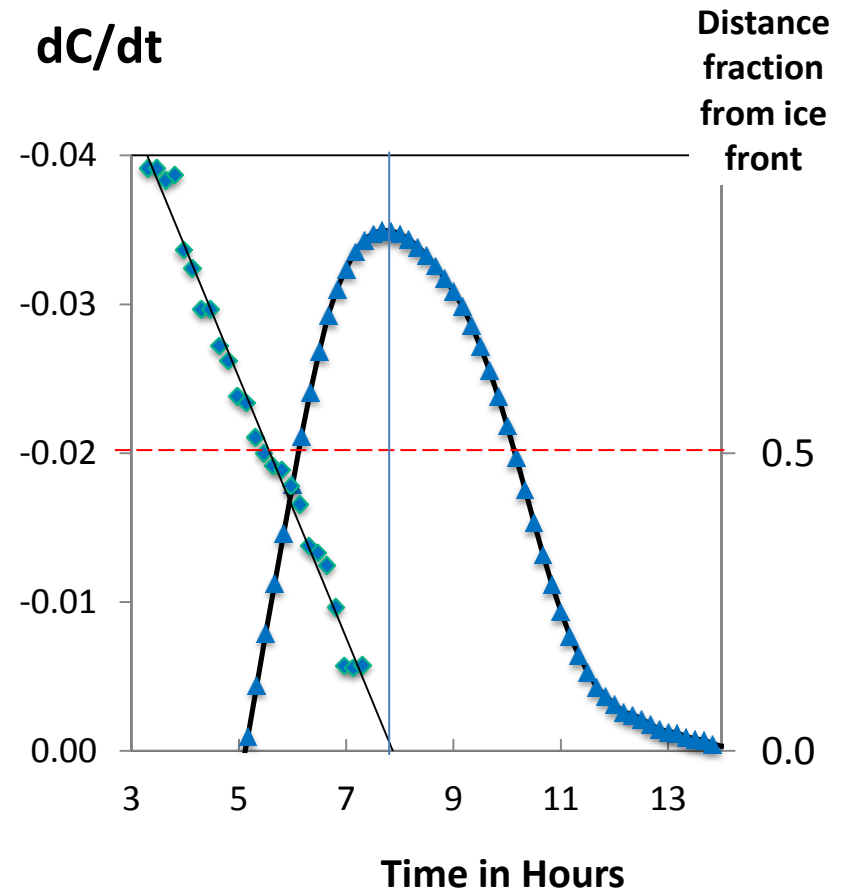
Lactose 3%

- In some cases the maximum in the derivative corresponds to the point at which the ice front has receded to 50% of the height of the product (in the case of lactose)



Sucrose 3%

- In other case the maximum in the derivative corresponds to the point at which the external ice front has receded to 100% of the height of the product (in the case of sucrose)
- Can these observation be used to indicate/inform the user about the flatness of the drying front?
- In all case the approach of the derivative to a value of zero indicates the end of the primary drying process.



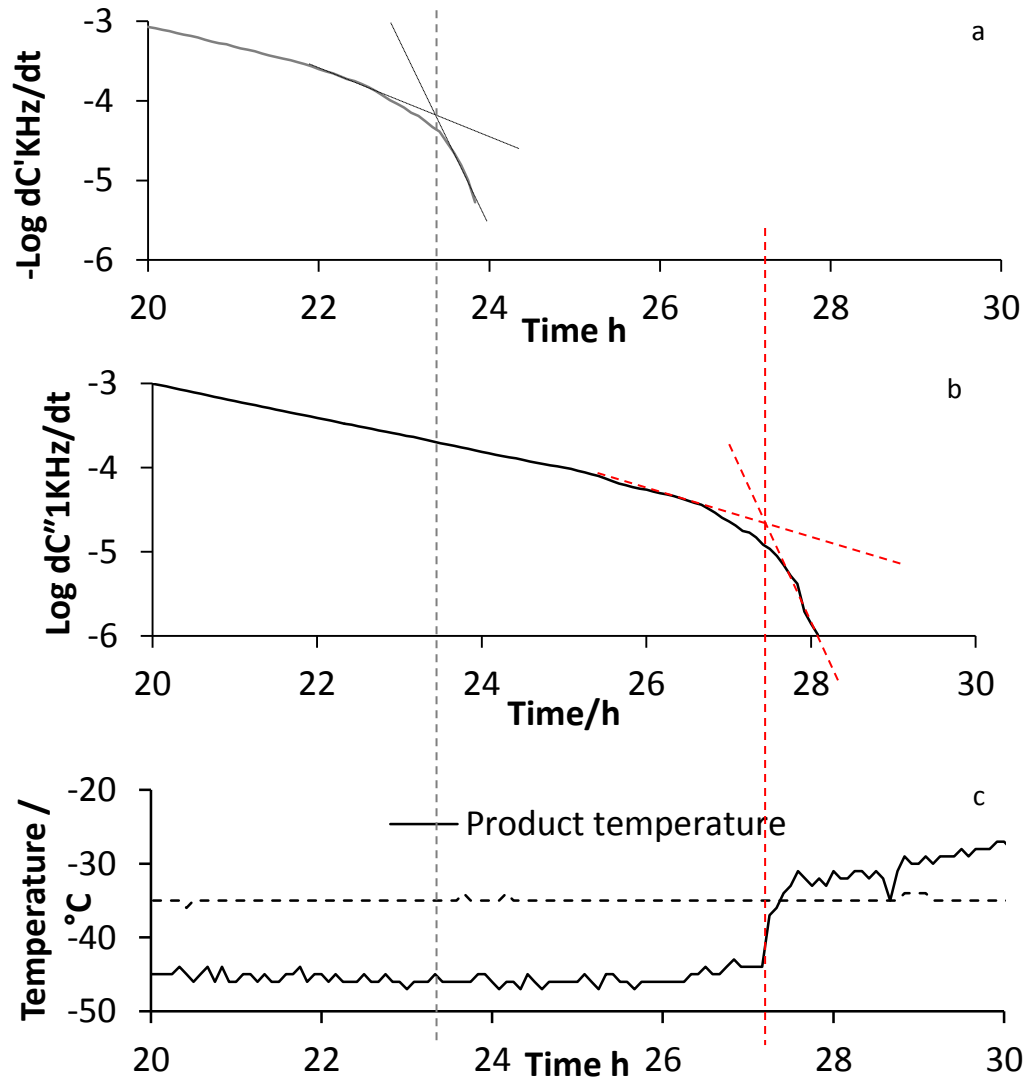
End Point Determination

- Impedance measurement data from sucrose 2.5% w/v were analysed for the determination of primary drying end point.
- Time slice of the imaginary capacitance at 1kHz showed a sharp decline as the ice sublimation was complete.



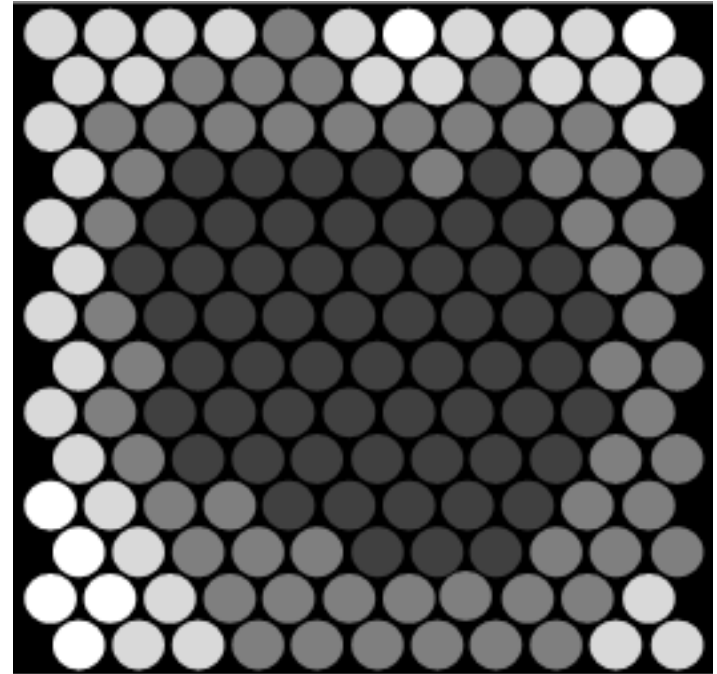
Results: Defining the End of Primary Drying

LyoDEA offers a non invasive measurement of primary drying time which is in good agreement with the thermocouple.



Shelf temperature distribution: Spatial mapping

- The temperature variation measurement during freezing stage.
- Thermocouple measurements of vials filled with oil.
- Gray scale shows minimum-maximum during freezing.
- $\Delta T \sim 1\text{-}2\text{ }^{\circ}\text{C}$ across shelf can affect ice formation (already stochastic) and impact drying time
- $1\text{ }^{\circ}\text{C}$ increase in 1° drying T can shorten drying time by $\sim 13\%$



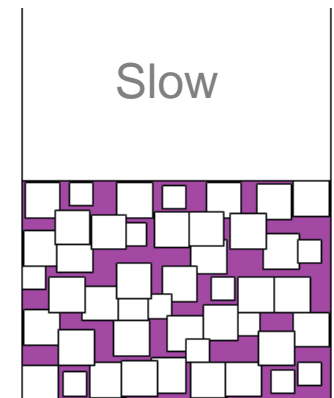
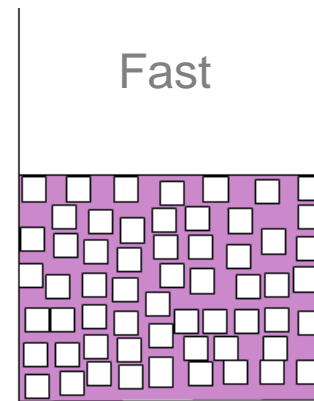
Impact of Spatial Temperature Map on Ice Formation

- Ice crystallization rates can impact the amount of ice and the particle size

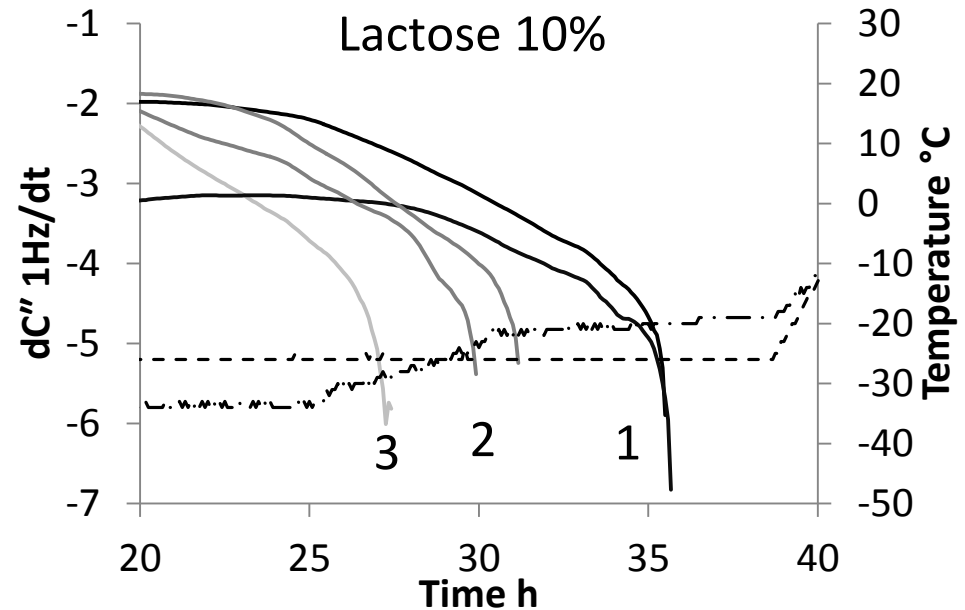
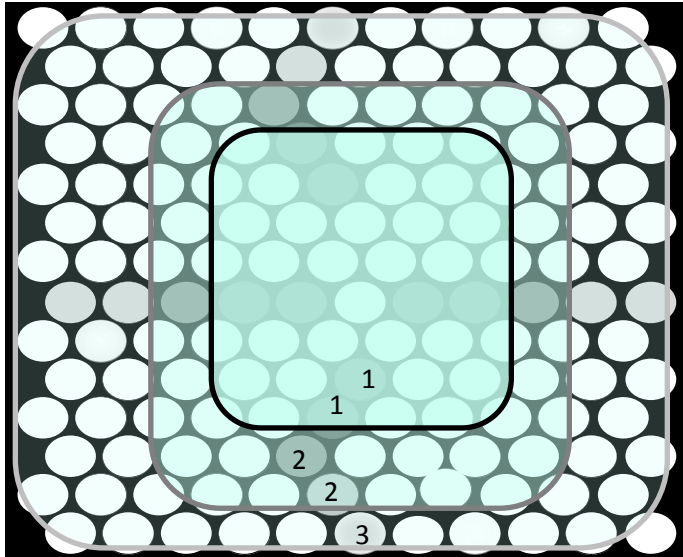
F Rate	Ice Crystal Size	R_p	Unfrozen fraction
Fast	Small	High	High
Slow	Large	Low	Low

Leading to variations in:

- drying rates (because of the impact on the resistance to vapour flow, R_p)
- concentration on solutes in the unfrozen fraction, which impacts T_g which impacts the primary drying temperature.



LyoDEA Spatial mapping: Primary drying times



1. Primary drying time distribution across the shelf identifies three distinct spatial regions characteristic of thermal variations in the shelf.
2. Edge effects – may extend across three vials around the periphery of the shelf

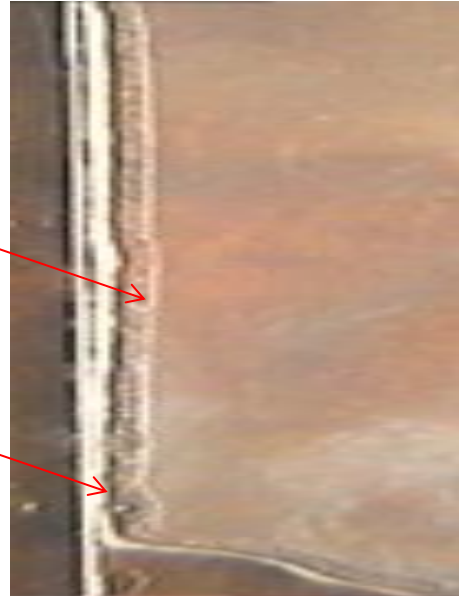


Product Collapse

- Conventionally measured by cryo-microscopic images

Sublimation front

Collapsed product



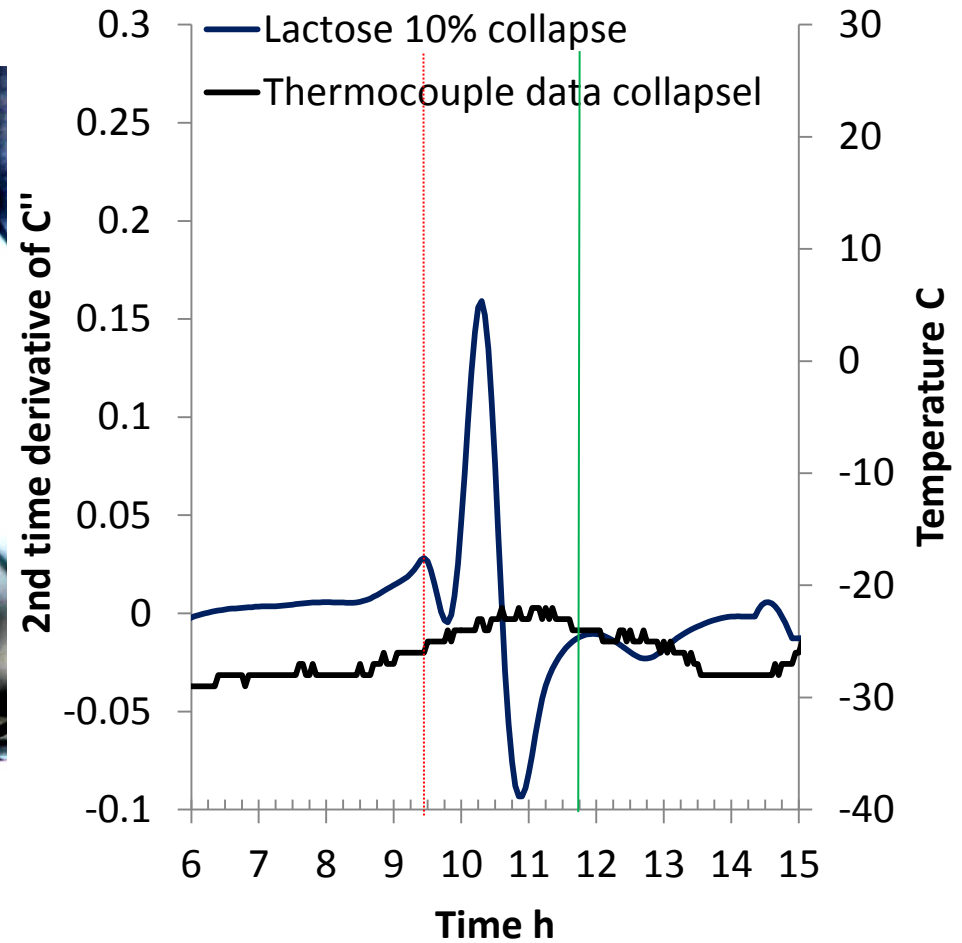
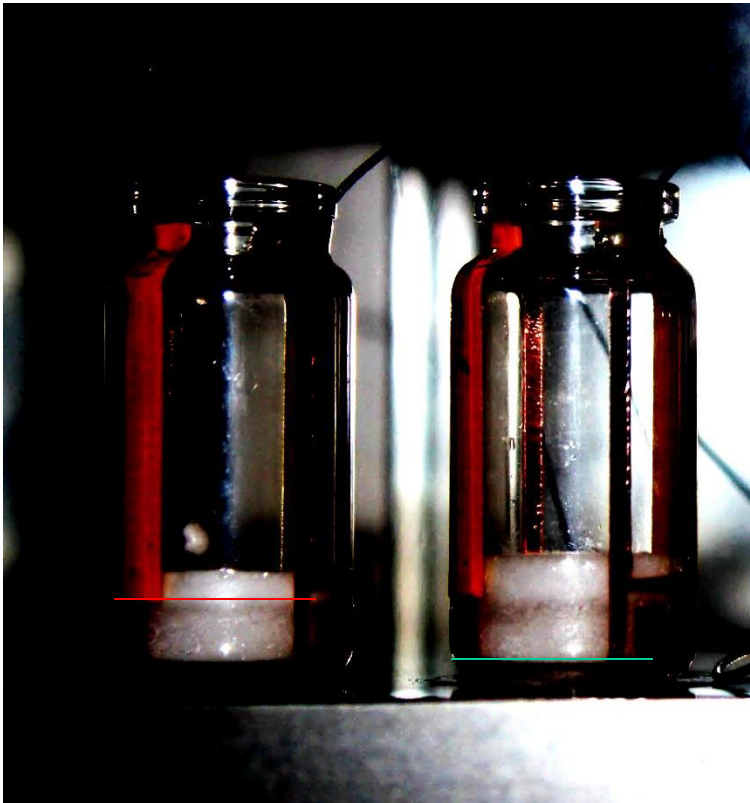
Microscopic images may not account for increase in the vapor pressure at sublimation front ,following increased resistance to vapor flow during the later stage of primary drying; **potentially vulnerable to collapse**

Collapse measurement within the real conditions may provide such information

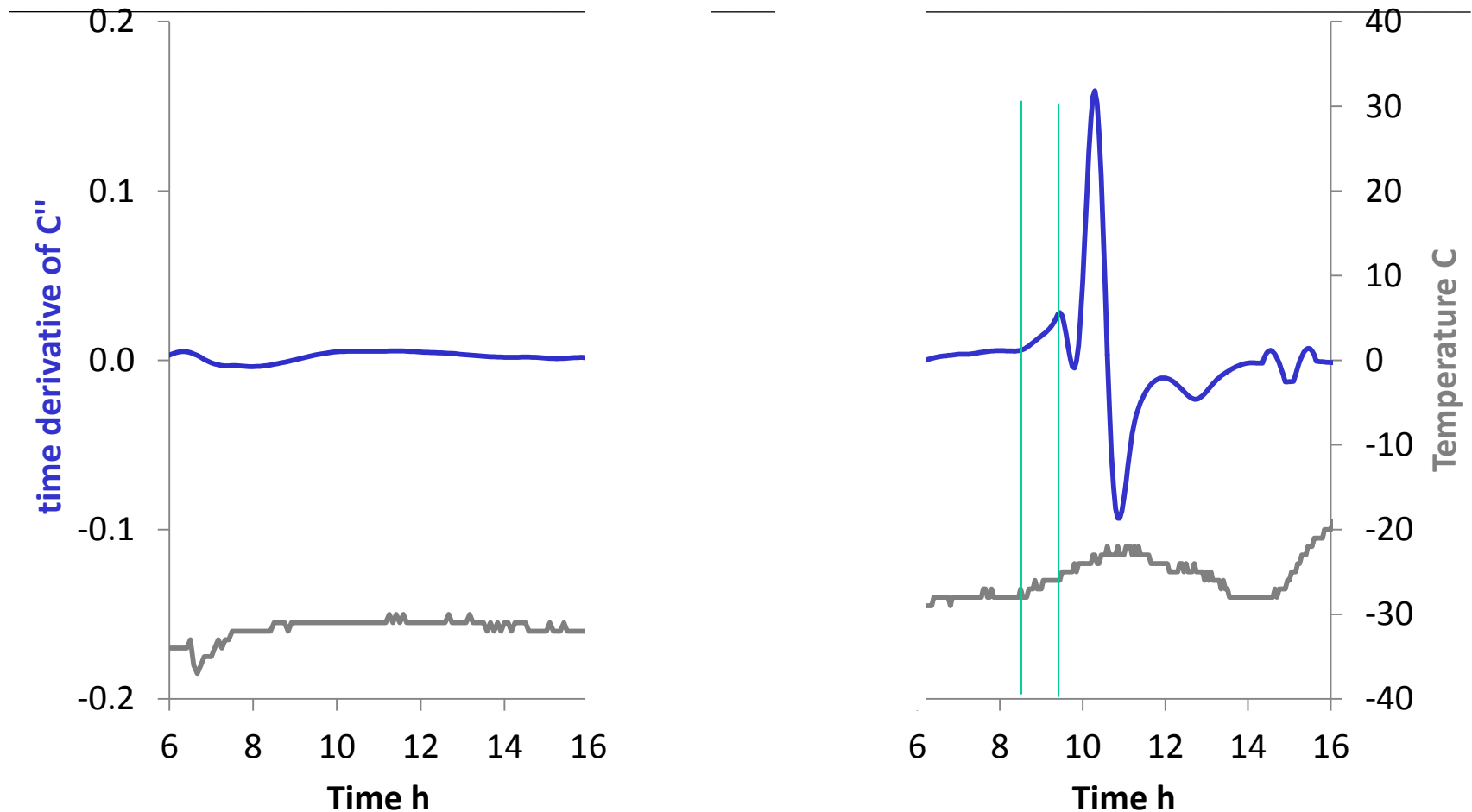


LyoDEA: Product collapse

A sharp spike in the capacitance profile at 1 kHz characterizes the product collapse



Lactose 10% Collapse : 2nd derivative



the capacitance profile of collapse free product (LEFT) was seen to be fairly uniform, un-like the collapsed product (RIGHT)



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